UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: March 20, 2012

SUBJECT: Developmental Neurotoxicity Positive Control Data Evaluation for Wil Research Laboratories (9 DERs attached)

PC Code:
600004 (IDPN)/111-94-4
600091 (D-Amphetamine sulfate)/51-63-8
600006 (Chlorpromazine HCl)/69-09-0
600008 (Acrylamide)/79-06-1
600007 (Trimethyltin Cl)/1066-45-1
600093 (Methimazole)/60-56-0
057810 (Propylthiouracil)/51-52-5

DP Barcode: D302810

Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0054597
MRID No.: see table I

Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: see PC Code list above
40 CFR: NA

Ver.Apr. 2010

FROM: Marion Copley, DVM, DABT 3/20/12
Science Information Management Branch
Health Effects Division, (7509P)

THROUGH: Jess Rowland, Acting Associate Director 4/1/12
Immediate Office
Health Effects Division (7509P)

TO: Brenda May, Branch Chief
Science Information Management Branch
Health Effects Division, (7509P)

I. CONCLUSIONS

The following DNT positive control data have been evaluated resulting in the following classifications.

MRID 45457501a: This **method validation** study is classified **Acceptable/Non-guideline. It does not however, constitute an adequate positive control study for motor activity. WIL Research Laboratory has optimized conditions for motor activity tests in postweaning (6 wk) Sprague-Dawley Crl:CD®(SD)IGS BR rats using SDI Photobeam Activity System for the time period around 1999 (in life period of study).** This study satisfies the purpose for which it was intended. **It does not however, constitute an adequate positive control study for motor activity.** This method validation study does not satisfy any guideline requirement.

MRID 45457501b: This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting startle response with habituation due to IDPN treatment in postweaning (4-7 wk) pups (in life time period unspecified). Although the WIL study report presented graphical data indicating ability to detect changes the study report does not meet present OPPTS 870.6300 guidelines. The: 1) in life dates of the study were absent. 2) There was no numerical data (individual animal and mean (SD) data) presented to support the graphical representation of the data. 3) Animal body weights were only summarized as means, range or individual animal weights were not presented.** This positive control study does not satisfy any guideline requirement.

MRID 45457501c: This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated proficiency in this study for detecting changes in Motor Activity tests in postweaning (6 wk) Sprague-Dawley Crl:CD®(SD)IGS BR rats due to d-Amphetamine sulfate or Chlorpromazine HCl treatment for the time period around 1999 (in life period of study). Although the WIL study report presented data indicating ability to detect changes, the study report does not meet present OPPTS 870.6200 or 870.6300 guidelines due to reporting deficiencies. 1) There was no individual animal data. 2) Interval data for the motor activity sessions (subsessions) need to be provided to demonstrate habituation.** This positive control study does not satisfy any guideline requirement.

MRID 45457501d: This **method validation** study is classified **Acceptable/Non-guideline. It does not however, constitute an adequate positive control study for motor activity. WIL Research Laboratory has demonstrated proficiency in testing acoustic startle response tests showing habituation and pre-pulse inhibition in preweaning (PND 20) Sprague Dawley Crl:CD®(SD)IGS BR rats using the SR-Lab Startle Response System for the time period around 1999 (in life period of study). This study does not constitute acceptable positive control data.** This method validation study does not satisfy any guideline requirement. The data from the morphometry study will provide historical control data for Sprague-Dawley rats (PND 11) at the Laboratory.

MRID 45457501e: This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) inter-observer reliability or proficiency in this study for detecting FOB changes due to IDPN treatment in rats (strain and age unspecified). The: 1) in life dates of the study were absent, 2) protocol details very limited, and 3) raw data were limited (sometimes only presented graphically). These reporting deficiencies limit the usefulness of the study to validate DNT studies.**

Presentation of the study dates, additional protocol detail (for example, details of the methods and scoring criteria for the FOB) and raw data for this study are necessary in order to confirm that the laboratory has proficiency for detecting the changes. A complete review of the data was not conducted. This positive control study does not satisfy any guideline requirement.

MRID 45457501f: This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting Motor Activity, FOB and neuropathology changes due to acrylamide treatment and neuropathology changes due to trimethyltin chloride (age of rats not specified) for the time period around 1991 (in life date). Although the WIL study report presented some tabular data indicating ability to detect FOB changes, the study report does not meet present requirements for positive control data needed for a developmental neurotoxicity study. 1) There were no details on the animals used in the study. 2) There were no numerical data (individual animal and mean (SD) data) presented to support the conclusions of the investigator for Motor activity, FOB or Neuropathology. 3) There were no details on the statistical methods used.** This positive control study does not satisfy any guideline requirement.

MRID 45457501g: This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting changes in FOB and Motor Activity tests in rats (age, strain and source are unspecified) due to d-Amphetamine sulfate or Chlorpromazine HCl treatment for the time period around 1990 (in life period of study). Although the WIL study report presented some numerical and graphical data indicating ability to detect changes, the results could not be confirmed due to the limited data presented (see deficiency section for details).** This positive control study does not satisfy any guideline requirement.

MRID 46779203: This study is classified **unacceptable/nonguideline. The reviewer can not determine whether WIL Research Laboratory can detect the effects discussed in the investigators' conclusions since there were no details regarding the methods, test animal, test material, individual animal data or summary data (other than several graphs). Based on the parameters in the graphs and those mentioned in the report's results section it appears that this study may be acceptable as positive control data if a complete report is submitted.**

MRID 46779204: This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting neurotoxicity changes due to PTU treatment in pre- and postweaning Crl:CD® (SD)IGS BR rat pups (time period not provided). Although WIL Research Laboratory staff did identify some developmental and behavioral deficits (FOB, motor activity, auditory startle, and learning and memory tests) the study report does not meet present OPPTS 870.6300 guidelines. The: 1) in life dates of the study were absent, 2) protocol details very limited, and 3) raw data were limited (sometimes only presented graphically). These reporting deficiencies limit the usefulness of the study to validate DNT studies. Presentation of the study dates, additional protocol detail (for example, details of**

the methods and scoring criteria for the FOB) and raw data for this study are necessary in order to confirm that the laboratory has proficiency for detecting the changes. This positive control study does not satisfy any guideline requirement.

See Table II for summaries of these studies

II. ACTION REQUESTED

Evaluate DNT positive control data for Wil Research Laboratories

III. RESULTS

Table I MRID summary table

MRID	Comments
45457501a	New DER
45457501b	New DER
45457501c	New DER
45457501d	New DER
45457501e	New DER
45457501f	New DER
45457501g	New DER
46779203	New DER
46779204	New DER

Table II DNT Positive Control Summary Table for Wil Research Laboratories (see key below for meaning of abbreviations)

MRID	Rpt Year	Test Cmpd	Neurobehavioural		Auditory Startle Habituation	Learning & Memory		NP/Brain	(In life year) Classification	Age tested/Comments
			FOB	Motor Act.		Water Maze	Pass./avoid.			
WIL		TXR 0054597								
45457501a	2001	none		x (method validation only)					(1999) A/NG	Post/SDI Photobeam Activity System validation
45457501b	2001	IDPN			x				(date?) U/NG	Post/
45457501c	2001	d-amphetamine SO4, chlorpromazine HCl		x					(1999) U/NG	Post/SDI-PAS
45457501d	2001	none			x (historical control & method validation only)			brain morph (historical control only)	(1999) A/NG	Pre/SR-Lab Startle Response System validation & morph hist. control
45457501e	2001	IDPN	x						(1991) U/NG	no age/no detail, or inter-observer reliability
45457501f	2001	acrylamide, trimethyltin chloride	x	x				NP	(1991) U/NG	no age/little detail
45457501g	2001	d-amphetamine SO4, chlorpromazine HCl	x	x					(1990) U/NG	no age/Digiscan micro
46779203	2006	Methimazole	x	x	x	Biel maze		NP, morph	(not given) U/NG	Pre, Post, YAd/
46779204	2001	PTU	x	x	x	Biel maze		brain morph	(2001) U/NG	Pre, Post/

KEY
x = neurologic parameter is tested
PTU = propylthiouracil
IDPN = iminodipropionitrile
NP = neuropathology
morph = morphometry
PNS = peripheral nervous system
Classification code
A/NG = acceptable non-guideline
U/NG = unacceptable non-guideline
AGE of Tested animals
Pre (weaning) = PND 4-21
Post (weaning) = 22-65 (3-9 wk old)
YAd (yound adult) = 65-75 (10-11 wk)
Ad (adult) = > 75 days (after 11 wk)

DATA EVALUATION RECORD

STUDY TYPE: NEUROTOXICITY POSITIVE CONTROL DATA IN RATS FROM WIL RESEARCH LABORATORIES
MRID 45457501e

Prepared for

Health Effects Division
U.S. Environmental Protection Agency
Office of Pesticide Programs
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Work Assignment No. 131-2006

Primary Reviewer: Carol S. Wood, Ph.D., D.A.B.T.	Signature: Date:	Carol S Wood MAY 11 2006
Secondary Reviewers: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	Sylvia S. Talmage MAY 11 2006
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross MAY 11 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:** Marion Copley
Science Information Mgmt. Branch, HED (7509P) **Date:** 3/20/12
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature:** Jess Rowland
Immediate Office, Health Effects Division (7509P) **Date:** 4/11/12
Template version 02/06

TXR#: 0054597

Abbreviated DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity Study - Rat; Positive Control, Non-guideline

PC CODE: 600004 **DP BARCODE:** D302810

TEST MATERIAL (PURITY): 3', 3'-Iminodipropionitrile (IDPN; 90% a.i.)

SYNONYMS: Iminodipropionitrile

CITATION: Pitt, J. (2001) Positive and Historical Control Data for Neurotoxicity Studies: An inter-observer reliability study of technicians assigned to perform F.O.B. observations for neurotoxicity studies. WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. WIL-99035, June 26, 2001. MRID 45457501e (pp 1-5, 91-93). Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a positive control neurotoxicity study (MRID 45457501e), 5 rats/sex/group were administered a single dose of IDPN (90% a.i., Batch# not given) by gavage at doses of 0 or 2000 mg/kg. Deionized water was used as the negative control; the test article was dosed neat. A functional observational battery (FOB) was conducted by eight trained technicians following the onset of clinical signs. No details of the FOB methods or age and strain of rat used were given.

No data were included with the report. In a short results section it was stated that findings for all endpoints of the FOB were consistent between technicians for both treated and control animals.

Based on insufficient data, it is not possible to determine whether the testing facility demonstrated proficiency in conducting the FOB or in inter-observer reliability.

This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) inter-observer reliability or proficiency in this study for detecting FOB changes due to IDPN treatment in rats (strain and age unspecified). The: 1) in life dates of the study were absent, 2) protocol details very limited, and 3) raw data were limited (sometimes only presented graphically). These reporting deficiencies limit the usefulness of the study to validate DNT studies. Presentation of the study dates, additional protocol detail (for example, details of the methods and scoring criteria for the FOB) and raw data for this study are necessary in order to confirm that the laboratory has proficiency for detecting the changes.** A complete review of the data was not conducted. This positive control study does not satisfy any guideline requirement.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided.

DATA EVALUATION RECORD

STUDY TYPE: REVIEW OF MOTOR ACTIVITY POSITIVE CONTROL DATA FROM WIL RESEARCH CORPORATION

MRID 45457501a

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131-2006

Primary Reviewer:
Sylvia S. Talmage, Ph.D., D.A.B.T.
Signature: Sylvia S. Talmage
Date: APR 13 2006

Secondary Reviewers:
Carol S. Wood, Ph.D., D.A.B.T.
Signature: Carol S Wood
Date: APR 13 2006

Robert H. Ross, M.S., Group Leader
Signature: Robert H. Ross
Date: APR 13 2006

Quality Assurance:
Lee Ann Wilson, M.A.
Signature: L. A. Wilson
Date: APR 13 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:**
Science Information Mgmt. Branch, HED (7509P) **Date:**
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature:**
Immediate Office, Health Effects Division (7509P) **Date:**

Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: Positive Control Data for Neurotoxicity Study – Method Validation Only (Motor Activity Test Session Duration) - Rat

PC CODE: NA **DP BARCODE:** D302810

TEST MATERIAL (PURITY): Not applicable (untreated animals)

SYNONYMS: Not applicable

CITATION: Pitt, J. (2001) Positive and Historical Control Data for Neurotoxicity Studies: Determination of test session duration and test interval for the San Diego photobeam activation system using untreated rats. WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. WIL-99140, June 26, 2001. MRID 45457501a (pp 1-5, 24-31). Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a motor activity positive control study (MRID 45457501a), a group of 24 six-week-old untreated Sprague-Dawley Crl:CD®(SD)IGS BR rats (12/sex) were monitored in an SDI Photobeam Activity System. The study objective was to determine the optimum duration of the test session and the test interval that would result in normal activity levels by the last 20% of the session following placement in a novel environment (habituation). The monitoring period was 120 minutes; counts were tallied in one- and five-minute intervals or subsessions. No other neurobehavioral procedures were performed.

Individual animal data were not presented and male and female data were combined. Using the one-minute subsessions, habituation was evident after 36 minutes. Based on five-minute subsessions, habituation was evident by 45 minutes. Therefore, a 60-minute motor activity test with five-minute subsessions is adequate for determining habituation in young untreated rats.

This **method validation** study is classified **Acceptable/Non-guideline. It does not however, constitute an adequate positive control study for motor activity. WIL Research Laboratory has optimized conditions for motor activity tests in postweaning (6 wk) Sprague-Dawley Crl:CD®(SD)IGS BR rats using SDI Photobeam Activity System for the time period around 1999 (in life period of study).** This study satisfies the purpose for which it was

intended. **It does not however, constitute an adequate positive control study for motor activity.** This method validation study does not satisfy any guideline requirement.

COMPLIANCE: The study was not subject to GLP, but was conducted in general accordance with GLP standards. No Quality Assurance inspections were performed. A Data Confidentiality statement was provided for the overall report.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Not applicable

2. **Vehicle control:** Not applicable

3. **Test animals:**

Species:	Rat	
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR	
Age/weight at dosing:	Approximately 6 weeks old/males: 225±75 g; females 135±25 g	
Source:	Available stock	
Housing:	Not described (per WIL Standard Operating Procedures)	
Diet:	Not described	
Water:	Not described	
Environmental conditions:	**Temperature:**	72±4°F
	Humidity:	50±20%
	Air changes:	Not described
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	5 days	

B. STUDY DESIGN:

1. **In life dates:** Start: April 12, 1999; End: April 13, 1999

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1. Method of assignment was not specified. The rats were untreated.

Table 1. Study design

Experimental parameter	Control
Total number of animals/sex	12/sex
Behavioral testing (motor activity)	12/sex

3. **Test substance preparation and analysis:** Not applicable

4. **Statistics:** Not applicable

C. METHODS / OBSERVATIONS:

1. **Neurobehavioral assessment – Locomotor activity:** Locomotor activity (total activity) of 24 untreated juvenile rats was monitored for 120 minutes to determine the test session duration and subsession length that would show habituation. Activity was monitored in a SDI Photobeam Activity System (San Diego Instruments, San Diego, CA). Each unit was a clear plastic box with seven photobeam detecting units. Activity was measured as interruptions of photobeams. Data were collected in 1-minute subsessions and summed as 5-minute subsessions. Animals were allowed to acclimate for one minute before monitoring

commenced. Monitoring took place in a sound proof room equipped with a white-noise generator set to operate at approximately 70 db.

2. **Positive controls:** Not applicable since this was a method validation study.

II. RESULTS – Motor Activity: Results were presented as mean activity counts for 1- and 5-minute intervals. The 5-minute subsession data are summarized in Table 2. Data for males and females were not presented separately. Habituation was evident by Subsession 8, 40-45 minutes. The 1-minute interval data showed habituation after 36 minutes.

TABLE 2. Mean motor activity counts (5-minute subsession data)

Subsession number	Counts
Subsession 1	49.8
Subsession 2	27.4
Subsession 3	12.2
Subsession 4	10.5
Subsession 5	10.1
Subsession 6	8.6
Subsession 7	4.3
Subsession 8	1.2
Subsession 9	0.8
Subsession 10	1.8
Subsession 11	1.3
Subsession 12	1.8
Subsession 13	3.7
Subsession 14	1.7
Subsession 15	1.6
Subsession 16	1.9
Subsession 17	1.4
Subsession 18	0.7
Subsession 19	1.1
Subsession 20	0.5
Subsession 21	3.1
Subsession 22	1.1
Subsession 23	0.8
Subsession 24	0.2

Data were extracted from Table 1, p. 31, MRID 45457501a.
Standard deviations were not calculated.
n= 12/sex (data are for males and females combined).

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** Untreated rats returned to normal locomotor activity levels after 36 minutes. The investigators concluded that a 45-minute session would be adequate to allow rats to fully habituate to a novel environment. However the investigators chose a 60-minute test session for future studies to allow for normal activity levels by the last 20% of the test session.

B. **REVIEWER COMMENTS:** The reviewer agrees with the investigators' conclusions, i.e., a 60-minute motor activity session with 5-minute subsessions is adequate to allow for full habituation of untreated young adult rats. The investigators demonstrated proficiency in conducting motor activity tests. The investigators should have presented data separately for males and females in accordance with neurotoxicity study guidelines.

WIL Research Laboratory has optimized conditions for motor activity tests in postweaning (6 wk) Sprague-Dawley Crl:CD®(SD)IGS BR rats using SDI Photobeam Activity System for the time period around 1999 (in life period of study). This study satisfies the purpose for which it was intended. **It does not however, constitute an adequate positive control study for motor activity.** This method validation study does not satisfy any guideline requirement.

C. **STUDY DEFICIENCIES:** There were no major study deficiencies considering the purpose of this study. However, individual animal data should have been provided and locomotor data should have been summarized separately for males and females. Although there were no individual animal data presented, this study satisfies the purpose for which it is intended. It does not constitute an adequate positive control study for motor activity however.

DATA EVALUATION RECORD

STUDY TYPE: REVIEW OF STARTLE RESPONSE POSITIVE CONTROL DATA FOR WIL RESEARCH LABORATORIES, INC.
MRID 45457501b

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131-2006

Primary Reviewer:
Sylvia S. Talmage, Ph.D., D.A.B.T.
Signature: Sylvia S. Talmage
Date: APR 13 2006

Secondary Reviewers:
Carol S. Wood, Ph.D., D.A.B.T.
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Date: APR 13 2006

Robert H. Ross, M.S., Group Leader
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Quality Assurance:
Lee Ann Wilson, M.A.
Signature: L.A. Wilson
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EPA Reviewer: Marion Copley, D.V.M., D.A.B.T.
Science Information Mgmt. Branch, HED (7509P)
EPA Secondary Reviewer: Jess Rowland, M.S.
Immediate Office, Health Effects Division (7509P)

Signature: [signature]
Date: 7/20/12
Signature: [signature]
Date: 4/11/12

Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity Positive Control Data for Startle Response - Rats; Non-guideline

PC CODE: 600004 **DP BARCODE:** D303132

TEST MATERIAL (PURITY): IDPN (3'-3' iminodipropionitrile)

SYNONYMS: Iminodipropionitrile

CITATION: Pitt, J. (2001) Positive and Historical Control Data for Neurotoxicity Studies: Demonstration of the sensitivity of the SR-Lab Startle Response System to detect chemical-induced alterations in acoustic startle response in rats. WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. WIL-99144, June 26, 2001. MRID 45457501b (pp 1-5, 32-55). Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a positive control startle response study (MRID 45457501b), groups of 4-5 week old Sprague-Dawley rats (8/sex/dose) were given an intraperitoneal injection of Iminodipropionitrile (IDPN, lot# 90906AT) for three consecutive days at doses of 0 (physiological saline), 200, or 400 mg/kg bw. Habituation of the acoustic startle response was tested in all animals 4 and 11 days after the last treatment, and pre-pulse inhibition of the acoustic startle response was tested in all animals 12 days after treatment. Startle response measurements were made using the SR-Lab Startle Response System. Testing was done in a sound-attenuated room equipped with a white-noise generator set to operate at approximately 70 db. No other observations or neurobehavioral tests were performed.

Data were presented graphically, separately for males and females. Habituation of the startle response was demonstrated for both sexes in the control and 200 mg/kg/day groups and at both 4 and 11 days post-treatment. Startle response (V_{max} and V_{avg}) was greatly reduced in males in the 400 mg/kg/day group on both testing days. Latency to the V_{max} (T_{max}) was increased on both testing days for males in the 400 mg/kg/day group. Females in the 400 mg/kg/day group also had decreased amplitude and increased latency on both days compared with the controls, but the magnitude of effect was not as great as that of the males, and some recovery to control levels was noted by day 11.

At 12 days after treatment, both sexes in the control and 200 mg/kg/day groups showed habituation with increasing intensity of the pre-pulse. Males in the 400 mg/kg/day group had a drastically reduced V_{max} and V_{avg} and greatly increased T_{max} with no change in the magnitude of effect with increasing pre-pulse intensity. Females in the 400 mg/kg/day group also had decreased amplitude and increased latency compared with controls with effects seen mainly for trials without a pre-pulse and at 70 db.

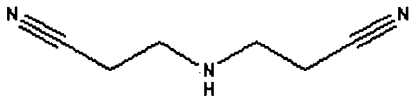
This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting startle response with habituation due to IDPN treatment in postweaning (4-7 wk) pups (in life time period unspecified). Although the WIL study report presented graphical data indicating ability to detect changes the study report does not meet present OPPTS 870.6300 guidelines. The: 1) in life dates of the study were absent. 2) There was no numerical data (individual animal and mean (SD) data) presented to support the graphical representation of the data. 3) Animal body weights were only summarized as means, range or individual animal weights were not presented.** This positive control study does not satisfy any guideline requirement.

COMPLIANCE: The study was not subject to GLP, but was conducted in general accordance with GLP standards. The raw data and draft report were audited by the WIL Quality Assurance Unit. A Data Confidentiality statement was provided for the overall report.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: IDPN

Description:	Not described (Aldrich Chemical Company, Milwaukee, WI)
Lot #:	90906AT
Purity:	Not provided.
CAS # of TGAI:	111-94-4
Structure:	

2. Vehicle: physiological saline

3. Test animals:

Species:	Rat	
Strain:	Sprague Dawley Crl:CD®(SD)IGS BR	
Age/weight at dosing:	Approximately 4-5 weeks/males, 154 g; females 129 g	
Source:	Not provided	
Housing:	Individually, per WIL Standard Operating Procedures	
Diet:	Not described	
Water:	Not described	
Environmental conditions:	**Temperature:**	72±4EF
	Humidity:	30-70%
	Air changes:	Not described
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Not provided	

B. STUDY DESIGN:

1. In life dates: Start: not provided; End: not provided

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1 by a computerized random sort program so that body weight means for each group were comparable. Rats were administered IDPN by intraperitoneal injection for three consecutive days. Individual doses were calculated based upon the animal's body weight prior to administration. Dosage volume was 5 mL/kg. Further details on dose preparation and dose selection were not provided. The control group was administered physiological saline.

TABLE 1. Study design

Experimental parameter	Dose group (mg/kg bw)		
	Control (0)	Low dose (200)	High dose (400)
Total number of animals/sex/group	8/sex	8/sex	8/sex
Behavioral testing (startle response)	8/sex	8/sex	8/sex

3. Test Substance preparation and analysis: Not described.

C. METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** No observations other than startle response were reported.

2. **Neurobehavioral assessment – Startle Response:** Startle response was tested in an SR LAB Startle Response System (San Diego Instruments, CA). Test subjects were placed in an acrylic cylinder mounted on a platform enclosed in a sound-attenuating chamber. A piezoelectric force-transducer mounted under the platform measured the startle response as a deflection of the platform. Deflection of the platform induced a voltage change in the piezoelectric force-transducer which was converted to a digital signal for analysis. Startle response evaluations were performed in a sound-attenuated room equipped with a white-noise generator set to operate at 65-70 db.

   The acclimation period in the chamber was five minutes. **Startle response** was measured 4 and 11 days after the last treatment. The startle stimulus was a 115-db mixed frequency noise burst of 20 ms in duration. Responses were recorded during the first 100 ms following the stimulus. The test session consisted of 50 trials with an eight-second inter-trial interval. Startle response data were analyzed in five blocks of 10 trials each plus all 50 trials. Startle response measures used to evaluate **habituation** were maximum response amplitude (V_{max}), average response amplitude (V_{avg}), and latency to V_{max} (T_{max}).

   Pre-pulse inhibition of the startle response was tested 12 days after the last treatment. Pre-pulse inhibition of the acoustic startle response was evaluated in a similar manner except that the pre-pulse stimulus was preceded by a less intense 20-ms signal. Pre-pulse trials consisted of pre-pulse intensities of 70, 75, 80, 85, and 90 db and a trial of no-prepulse for a total of six trials. Each trial type was presented eight times for a total of 48 trials. The order of trial types was balanced using a Latin-square design; the first trial was always a no-prepulse trial. Pre-pulse inhibition response was analyzed in six trial blocks by pre-pulse intensity.

D. DATA ANALYSIS:

1. **Statistical analyses:** The report stated that: The startle response activity data were analyzed using a two-way repeated measure of analysis (ANOVA) with trial as the within-subject factor. Significant treatment or treatment-time interactions ($p<0.05$) were subjected to a one-way ANOVA at each time point. Significant effects at a given time point were then subjected to Dunnett's test to determine significant treatment differences from the control group. These methods of analysis appear adequate.

II. RESULTS:

A. **AUDITORY STARTLE INHIBITION:** Data were only presented graphically as totals and means for blocks of ten trials, separately for males and females. **Habituation** was evident in control and low dose males and females on both testing days. High-dose males had

drastically reduced V_{max} and V_{avg} values and greatly increased T_{max} values on both testing days. High-dose females also had decreased amplitude and increased latency on both days compared with controls, but the magnitude of effects was not as great as that in the males. Some recovery to control levels was noted in females by day 11.

B. **PRE-PULSE INHIBITION:** Data were only presented graphically as the mean for each pre-pulse intensity, separately for males and females. Habituation was evident in control and low dose males and females with increasing intensity of the pre-pulse. High-dose males had a drastically reduced V_{max} and V_{avg} values and greatly increased T_{max} values with no change in the magnitude of effect with increasing pre-pulse intensity. High-dose females also had decreased amplitude and increased latency compared with controls with effects seen mainly in trials without a pre-pulse and at 70 db.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the SR-LAB Startle Response System is capable of detecting test article-induced changes in the V_{max}, V_{avg}, and T_{max} of the acoustic startle response. Pre-pulse inhibition of the startle response was demonstrated in the control group and the group treated for three days with 200 mg/kg/day. The 400 mg/kg/day group did not respond to the pre-pulse inhibition of the acoustic startle response, indicating chemically-induced changes in pre-pulse inhibition can be detected.

B. **REVIEWER COMMENTS:** The reviewer agrees that treatment-related changes in auditory startle habituation and pre-pulse inhibition of startle appears to be demonstrated. However it is not possible to confirm this conclusion in the absence of individual animal data and means (±SD). It was not possible to evaluate a dose-response since no effects occurred at the low dose whereas marked effects were found at the high dose. The magnitude of effects in males in the 400 mg/kg/day group did not appear to change over time. Males appeared to be more sensitive than females in both tests. **It could not be determined** whether the testing laboratory demonstrated proficiency in conducting the test.

C. **STUDY DEFICIENCIES:** The study report does not meet present OPPTS 870.6300 guidelines. There were major reporting deficiencies: Individual animal data along with means (±SD) were not provided. In life dates of the study were also not available. Animal weights were presented as means, no range or individual animal weights.

DATA EVALUATION RECORD

STUDY TYPE: SENSITIVITY OF SDI-PAS TO DETECT ALTERATIONS LOCOMOTOR ACTIVITY IN RATS by WIL LABORATORIES

MRID 45457501c

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131-2006

Robert H. Ross

Primary Reviewer:
Tom C. Marshall, Ph.D., D.A.B.T. Signature: for T. C. Marshall
Date: MAY 11 2006

Secondary Reviewers:
Carol S. Wood, Ph.D., D.A.B.T. Signature: Carol S Wood
Date: MAY 11 2006

Robert H. Ross, M.S., Group Leader Signature: Robert H. Ross
Date: MAY 11 2006

Quality Assurance:
Lee Ann Wilson, M.A. Signature: L. A. Wilson
Date: MAY 11 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:**
Science Information Mgmt. Branch, HED (7509P) **Date:** 3/20/12
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature:**
Immediate Office, Health Effects Division (7509P) **Date:** 4/11/12

Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity Positive Control Data for demonstrating sensitivity of locomotor activity - Rats; Non-guideline

PC CODE: 1) 600091; 2) 600006 **DP BARCODE:** D302810

TEST MATERIAL (PURITY): 1) d-Amphetamine sulfate; 2) Chlorpromazine hydrochloride (purities not provided)

SYNONYMS: Not applicable

CITATION: Pitt, J. (2001) Positive and Historical Control Data for Neurotoxicity Studies: Demonstration of the sensitivity of the SDI-PAS to detect alterations in locomotor activity in rats. Aventis Report No. WIL-2001-1; WIL Report No. WIL-99149. WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. June 26, 2001. MRID 45457501c (pp 1-5, 56-70). Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a motor activity positive control study (MRID 45457501c), five groups of 6 week old Sprague-Dawley Crl:CD®(SD)IGS BR rats (12/sex/group) were administered 0, 2 or 4 mg/kg of **d-Amphetamine sulfate** (ip injection) or 5 or 10 mg/kg of **Chlorpromazine HCl** (ip) then tested in a SDI Photobeam Activity System.

d-Amphetamine sulfate-treated male and female rats demonstrated a dose-dependent increase in total motor activity counts compared to controls. No interval data were provided. **Chlorpromazine HCl** caused a dose-dependent decrease in total activity counts in both males and females that was statistically significant at both dose levels ($p<0.01$). No interval data were provided.

This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated proficiency in this study for detecting changes in Motor Activity tests in postweaning (6 wk) Sprague-Dawley Crl:CD®(SD)IGS BR rats due to d-Amphetamine sulfate or Chlorpromazine HCl treatment for the time period around 1999 (in life period of study). Although the WIL study report presented data indicating ability to detect changes, the study report does not meet present OPPTS 870.6200 or 870.6300 guidelines due to reporting deficiencies. 1) There was no individual animal data. 2) Interval data for the**

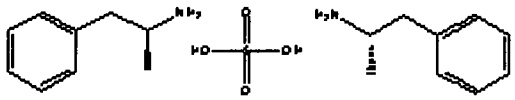
motor activity sessions (subsessions) need to be provided to demonstrate habituation. This positive control study does not satisfy any guideline requirement.

COMPLIANCE: No Quality Assurance or Data Confidentiality statements were provided.

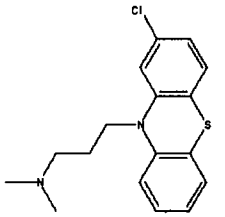
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: d-Amphetamine sulfate

Description:	Not described
Lot/batch #:	Research Biochemicals International. Natick, MA Lot No. BS-111-100
Purity:	Not provided
CAS # of TGAI:	51-63-8
Structure:	

2. Test material: Chlorpromazine hydrochloride

Description:	Not described
Lot/batch #:	Sigma Chemical Co., St. Louis, MO. Lot No. 48H1403
Purity:	Not provided
CAS # of TGAI:	69-09-0
Structure:	

3. Vehicle: Physiological saline

4. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR	
Age/weight at dosing:	Approximately 6 weeks old/males: 175±27 g; females 146±20 g	
Source:	Not provided	
Housing:	Not described (per WIL Standard Operating Procedures)	
Diet:	Not described	
Water:	Not described	
Environmental conditions:	**Temperature:**	72±4°F
	Humidity:	50±20%
	Air changes:	Not described
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	13 days	

B. STUDY DESIGN:

1. In life dates: Started: May 17, 1999; **Ended**: Mar 21, 1999

2. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 1. The report stated that "the rats were randomly assigned to treatment groups by body weight stratification in a block design, and subsequently allocated to group- and sex-uniform replicates for the locomotor activity evaluations." Dose selection was not discussed.

TABLE 1. Study Design			
Experimental parameter	d-Amphetamine Sulfate Dose (mg/kg)		
	Control (0)	2	4
Total No. of animals assigned	24	24	24
Motor activity	12/sex	12/sex	12/sex
	Chlorpromazine HCl Dose (mg/kg)		
	5.0	10	
Total No. of animals assigned	24	24	
Motor activity and FOB	12/sex	12/sex	

Data obtained from page 58, MRID 45457501c

3. **Reference compound dose preparation and analysis:** Single doses of **d-Amphetamine sulfate** were given to rats by intraperitoneal (ip) injection at 0.0 (1 mL/kg saline), 2 or 4 mg/kg. Similarly, single ip injections of **Chlorpromazine HCl** were given to rats at dose levels of 5 or 10 mg/kg. No analyses of the dose preparations were described.

C. METHODS / OBSERVATIONS:

Neurobehavioral assessment – Motor activity: A 60 minute pretest session was conducted 4-5 days prior to dosing with the reference compounds. Motor activity testing was then conducted 15 minutes after dosing. Motor activity was monitored in a SDI Photobeam Activity System (San Diego Instruments, San Diego, CA). Each unit was a clear plastic box with seven photobeam detecting units. Activity was measured as interruptions of photobeams. The test sessions were 60 minutes and the data were collected in 5-minute test intervals. Ambulatory activity (gross movements) and total activity (ambulatory plus stereotypic movements) were recorded separately. Monitoring took place in a sound proof room equipped with a white-noise generator set to operate at approximately 70 db.

D. DATA ANALYSIS:

Statistical analyses: Motor activity data were analyzed using the Analysis of Variance Test (ANOVA) followed by Dunnett's Test to determine the difference from control rats at significance levels of $p<0.05$ and $p<0.01$.

II. RESULTS – MOTOR ACTIVITY:

Total activity data are shown in Table 2. **d-Amphetamine sulfate** treatment caused dose-dependent increases in total (ambulatory) activity counts compared to controls in both males [103% (135%), 166% (193%) from low to high, respectively] and females [37% (46%), 146% (184%) from low to high, respectively] at 15 minutes post-treatment ($p<0.01$). The increase was significant in both sexes at the high dose, and in males at the low dose. **Chlorpromazine** treatment caused dose-dependent decreases in total (ambulatory) activity counts that were statistically significant in both males [77% 85% 96% 98% from low to high, respectively] and females [88% (92%), 97% (99%) from low to high, respectively] at 15 minutes post-treatment ($p<0.01$). No interval data were presented in the study report.

TABLE 2. Mean (±S.D.) motor activity data (total activity counts for session) [a]					
Test day	Dose (mg/kg)				
	Control	2 AMP	4 AMP	5 CPZ	10 CPZ
Males					
Pretest	1019±398.2	934±447.5	1246±735.0	1163±377.1	1221±444.6
Day 0	794±293.6	1609±655.8**	2110±638.9**	184±195.6**	33±25.6**
Females					
Pretest	1033±463.6	1190±543.2	974±343.2	853±231.6	1347±695.0
Day 0	1223±676.1	1672±759.8	3005±773.4**	142±116.9**	31±45.4**

[a] Data obtained from pages 61-62 in the study report (MRID 45457501c).
N = 12
* Statistically different from control, $p<0.05$; ** Statistically different from control, $p<0.01$
AMP = d-amphetamine sulfate; CPZ = chlorpromazine GCl

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The authors concluded that the motor activity testing system employed at their laboratory is capable of detecting both increases and decreases in motor activity.

B. REVIEWER COMMENTS:

The reviewer agrees that the data appears to support the investigators' conclusions. There was a dose-response relationship shown in both the d-amphetamine sulfate- and chlorpromazine HCl-treated rats, which demonstrated the laboratory's ability to detect alterations in motor activity. However there were several major reporting deficiencies that preclude being able to confirm the facilities results. See the deficiencies section for details.

WIL Research Laboratory has not demonstrated proficiency in this study for detecting changes in Motor Activity tests in postweaning (6 wk) Sprague-Dawley Crl:CD®(SD)IGS BR rats due to d-Amphetamine sulfate or Chlorpromazine HCl treatment for the time period around 1999 (in life period of study). Although the WIL study report presented data indicating ability to detect changes, the study report does not meet present OPPTS 870.6300 guidelines due to reporting deficiencies. 1) There were no individual animal data. 2) There was no interval data for the motor activity sessions need to be provided to demonstrate habituation.

C. STUDY DEFICIENCIES:

The study has several major reporting deficiencies. There were no individual animal data and the interval data for the motor activity sessions need to be provided to demonstrate habituation.

DATA EVALUATION RECORD

STUDY TYPE: REVIEW OF Historical Control/ Validation Study of the SR-LAB System in Young (PND 20) Rats

MRID 45457501d

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131-2006

Primary Reviewer: Tom C. Marshall, Ph.D., D.A.B.T.	Signature: for T.C. Marshall Date: MAY 11 2006
Secondary Reviewers: Carol S. Wood, Ph.D., D.A.B.T.	Signature: Carol S Wood Date: MAY 11 2006
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: MAY 11 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L.A. Wilson Date: MAY 11 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:**
Science Information Mgmt. Branch, HED (7509P) **Date:** 3/20/12
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature:**
Immediate Office, Health Effects Division (7509P) **Date:** 4/1/12

Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: Historical Control Data for Startle Response, Brain Morphometry - Rats OPPTS 870.6200a [§81-8]; OECD 424.

PC CODE: NA **DP BARCODE:** D302810

TEST MATERIAL (PURITY): None (no test article)

SYNONYMS: NA

CITATION: Pitt, J. (2001) Positive and Historical Control Data for Neurotoxicity Studies: WIL-99168: Historical Control/Validation Study of the SR-LAB System in Young (PND 20) Rats. Aventis Report No. WIL-99168). WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. June 26, 2001. MRID 45457501d (pp 1-5, 71-90). Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY:

Studies were conducted on untreated Sprague Dawley Crl:CD®(SD)IGS BR rats to provide historical control data for auditory startle habituation and brain morphometry (MRID 45457501d). Habituation of the acoustic startle response was tested in rats on PND 20 (two rats/sex/litter from five litters). Startle response measurements were made using the SR-Lab Startle Response System in a sound-attenuated room. Data were presented graphically, separately for males and females. Habituation of the startle response and pre-pulse inhibition of the startle response were demonstrated in both sexes.

Brain morphometry measurements were obtained but not presented on one pup/sex from six litters (PND 11) for eleven defined brain regions. The data from the study will provide historical control data for Sprague-Dawley rats at the Laboratory.

This **method validation** study is classified **Acceptable/Non-guideline. It does not however, constitute an adequate positive control study for motor activity. WIL Research Laboratory has demonstrated proficiency in testing acoustic startle response tests showing habituation and pre-pulse inhibition in preweaning (PND 20) Sprague Dawley Crl:CD®(SD)IGS BR rats using the SR-Lab Startle Response System for the time period around 1999 (in life**

period of study). This study does not constitute acceptable positive control data. This method validation study does not satisfy any guideline requirement. The data from the morphometry study will provide historical control data for Sprague-Dawley rats (PND 11) at the Laboratory.

COMPLIANCE: The study was not subject to GLP, but was conducted in general accordance with GLP standards. The raw data and draft report were audited by the WIL Quality Assurance Unit. A Data Confidentiality statement was provided for the overall report.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** None

2. **Vehicle:** NA

3. **Test animals:**

Species:	Rat	
Strain:	Sprague Dawley Crl:CD®(SD)IGS BR	
Age/avg. weight at testing:	PND 20; males, 47±9 g; females 40±10 g	
Source:	Not provided	
Housing:	Individually, per WIL Standard Operating Procedures	
Diet:	Not described	
Water:	Not described	
Environmental conditions:	**Temperature:**	72±4°F
	Humidity:	30-70%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Not provided	

B. STUDY DESIGN:

1. **In life dates:** Not provided

2. **Animal assignment and treatment:** The method of animal assignment was not described for any of the three studies (startle response habituation; startle response pre-pulse inhibition, brain morphometrics). Litters used in the study were born of dams bred at WIL Research Laboratories using their standard operating procedures.

3. **Test Substance preparation and analysis:** NA

4. **Statistics:** Statistical analyses were not conducted for either the auditory startle response tests or the morphometry study.

C. METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** The rats were observed for mortality/moribundity and general condition twice daily.

2. Neurobehavioral assessment:

a. Startle response: Startle response was tested in an SR LAB Startle Response System (San Diego Instruments, CA). Test subjects were placed in an acrylic cylinder mounted on a platform enclosed in a sound-attenuating chamber. A piezoelectric force-transducer mounted under the platform measured the startle response as a deflection of the platform. Deflection of the platform induced a voltage change in the piezoelectric force-transducer which was converted to a digital signal for analysis. Startle response evaluations were performed in a sound-attenuated room equipped with a white-noise generator set to operate at approximately 70 db.

Startle response habituation was evaluated for two rats/sex from five different litters on PND 20. The acclimation period in the chamber was five minutes. The startle stimulus was a 115-db mixed frequency noise burst of 20 ms in duration. Responses were recorded during the first 100 ms following the stimulus. The test session consisted of 50 trials with an eight-second inter-trial interval. Startle response data were analyzed in five blocks of 10 trials each plus all 50 trials. Startle response measures used to evaluate habituation were maximum response amplitude (V_{max}), average response amplitude (V_{avg}), and latency to V_{max} (T_{max}).

Pre-pulse inhibition of the acoustic startle response was evaluated in a similar manner except that a pre-pulse stimulus (less intense 20-ms signal) preceded the startle stimulus. Pre-pulse trials consisted of pre-pulse intensities of 70, 75, 80, 85, and 90 db and a trial of no-prepulse for a total of six trials. Each trial type was presented eight times for a total of 48 trials. The order of trial types was balanced using a Latin-square design; the first trial was always a no-prepulse trial. Pre-pulse inhibition response was analyzed in six trial blocks by pre-pulse intensity. The report refers to pre-pulse inhibition of the startle response being tested 12 days after the last treatment, but no treatment is specified in the study document. The sponsor needs to clarify this statement.

b. Morphometry: Measurements were obtained on one pup/sex from six litters (PND 11) for the following 11 defined brain regions:

1. Width of hemisphere, perpendicular to height and adjacent to top of dorsal white matter tract.
2. Height of hemisphere, immediately adjacent to medial margin of lateral ventricle.
3. Frontoparietal cortex thickness (vertical), parallel to height measurement.
4. Width of caudoputamen of hemisphere
5. One-half the width of the base of the fornix.
6. Vertical height from the base of the brain to the anterior commissure.
7. Vertical thickness of the frontoparietal cortex measured at the highest point of the corpus callosum.
8. Radial thickness of the parietal cortex (approximate 10 o'clock position).
9. Thickness of the piriform cortex (measured at surface indentation).
10. Thickness of the corpus callosum at the midline.
11. Height of the cerebellum (highest dimension), parallel to the thickness of the pons.

II. RESULTS:

A. OBSERVATIONS:

No deaths or signs of adverse health were observed in any animal in the three studies.

B. AUDITORY STARTLE HABITUATION:

Data were presented graphically as totals and means for blocks of ten trials, separately for males and females. The expected habituation was evident in both males and females. V_{max} and V_{avg} values decreased with successive trials. T_{max} appeared unaffected across the trials.

C. PRE-PULSE INHIBITION:

Data were presented graphically as the mean for each pre-pulse intensity, separately for males and females. Inhibition of the response as evidenced by decreased V_{max} was more dramatic in both sexes with each increase of the pre-pulse stimuli intensity as expected. T_{max} appeared unaffected across the trials.

D. MORPHOMETRY:

The data from the morphometry study are shown in Table 1. The authors stated that the measurements were repeatable across hemispheres, but these data were not shown.

TABLE 1. Mean (±SD) morphometric data [a]

Parameter	Dose (mg/kg/day)	
	Male	Female
PND 11		
1. Width of hemisphere	0.77±0.05	0.78±0.06
2. Height of hemisphere	0.22±0.04	0.21±0.02
3. Frontoparietal cortex thickness	0.16±0.01	0.15±0.01
4. Width of caudoputamen	1.72±2.84	2.05±3.42
5. Width of the base of the fornix	0.11±0.01	0.10±0.01
6. Vertical height from the base of the brain	0.06±0.01	0.05±0.01
7. Vertical thickness of the frontoparietal cortex	0.13±0.02	0.14±0.03
8. Radial thickness of the parietal cortex	0.30±0.06	0.32±0.04
9. Thickness of the piriform cortex	2.12±3.37	2.12±3.36
10. Thickness of the corpus callosum	3.83±1.98	3.83±1.98
11. Height of the cerebellum	0.39±0.07	0.36±0.06

[a] Data obtained from page 78, MRID 45457501d.
N =6, except for value #4 in males N=5

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators concluded that the SR-LAB Startle Response System is capable of measuring the V_{max}, V_{avg}, and T_{max} of the acoustic startle response in weanling rats in the age range of about PND 20. Similarly, pre-pulse inhibition of the startle response was demonstrated in weanling rats in the age range of about PND 20. The measurements across brain hemispheres were found to be repeatable in the morphometry study, but the data were not provided.

B. REVIEWER COMMENTS:

The reviewer agrees with the investigators' conclusions. Auditory startle habituation and pre-pulse inhibition of the startle response were demonstrated in weanling Sprague-Dawley rats. The testing laboratory demonstrated proficiency in conducting the tests. The data from the brain morphometry study provide historical control data for Sprague-Dawley rats at the Laboratory. Since the data on the measurements across hemispheres were not provided, their repeatability could not be evaluated.

WIL Research Laboratory has demonstrated proficiency in testing acoustic startle response tests showing habituation and pre-pulse inhibition in preweaning (PND 20) Sprague Dawley Crl:CD®(SD)IGS BR rats using the SR-Lab Startle Response System for the time period around 1999 (in life period of study). It does not however, constitute an adequate positive control study for motor activity. This method validation study does not satisfy any guideline requirement. The data from the morphometry study will provide historical control data for Sprague-Dawley rats (PND 11) at the Laboratory.

The deficiencies noted below do not limit the usefulness of this study for **method validation**, however they would need to be fixed if this were an actual positive control study.

C. STUDY DEFICIENCIES:

Raw data were not provided for the startle response studies, or for morphometry measurements across the brain hemispheres. The report refers to pre-pulse inhibition of the startle response being tested 12 days after the last treatment, but no treatment is specified in the study document. The sponsor needs to clarify this statement.

DATA EVALUATION RECORD

STUDY TYPE: REPEATED DOSE NEUROTOXICITY STUDY OF ACRYLAMIDE IN RATS by WIL LABORATORIES

MRID 45457501f

Prepared for

Health Effects Division,
Office of Pesticide Programs
US Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131-2006

Primary Reviewer: Tom C. Marshall, Ph.D., D.A.B.T.	Signature: Date:	Robert H. Ross for T. C. Marshall MAY 11 2006
Secondary Reviewers: Carol S. Wood, Ph.D., D.A.B.T.	Signature: Date:	Carol S Wood MAY 11 2006
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross MAY 11 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson MAY 11 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:** Marion Copley
Science Information Mgmt. Branch, HED (7509P) **Date:** 3/20/12
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature:**
Immediate Office, Health Effects Division (7509P) **Date:** 4/11/12
Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: 1) Repeated dose neurotoxicity study of Acrylamide in rats
2) Single dose neurotoxicity study of trimethyltin chloride in rats

PC CODE: 1) 600008
2) 600007

DP BARCODE: D302810

TEST MATERIAL (PURITY): 1) Acrylamide (99%)
2) Trimethyltin chloride (% ai not provided)

SYNONYMS: None

CITATION: Pitt, J. (2001). Positive and Historical Control Data for Neurotoxicity Studies: 1) Repeated dose neurotoxicity study of acrylamide in rats. 2) An acute neurotoxicity study of trimethyltin chloride in rats. (Aventis Report No. WIL-2001-1; WIL Report No. WIL-99034). WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. June 26 2001. MRID 45457501f (pp 1-5, 94-102). Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a positive control study (MRID 45457501f):
1) four groups of 24 rats (12/sex/group) were administered 0, 5, 10 or 20 mg/kg/day of **acrylamide** (99%) 5 days/wk for 4 weeks (route not specified). Functional Observational Battery (FOB) testing was performed 1 hr to 28 days after the first dosing. Motor activity testing was conducted at the end of the treatment period (methods not described). A histological examination for peripheral and central nervous system pathology was conducted on 6 rats/sex/group from the control and high dose groups.

2) one group of 10 rats (5/sex) were administered (7.5 mg/kg) a single intraperitoneal injection of **trimethyltin chloride**. Fifteen days after dosing the animals were euthanized and histopathology examined as for acrylamide above.

Acrylamide: The FOB results were presented in tabular form as dose group affected, but without incidence data. **Acrylamide**-treated male and female rats from the 10 and 20 mg/kg/day treatment groups demonstrated differences relative to controls during the 14, 21, and 28 day

evaluations, which became more apparent with each successive time point. Biologically significant lesions indicative of neurotoxicity were observed in the **acrylamide**-treated rats (20 mg/kg). Differences as compared to controls were described in the report, but the data were not provided to evaluate.

Trimethyltin chloride: The report stated that there was neuronal loss in the dentate gyrus and one of the male rats exhibited chromatolysis in the gasserian ganglion neurons.

Insufficient data are presented in the study report to assess proficiency in conducting tests on neurotoxicants like acrylamide and observing changes in the FOB and nervous system histology.

This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting Motor Activity, FOB and neuropathology changes due to acrylamide treatment and neuropathology changes due to trimethyltin chloride (age of rats not specified) for the time period around 1991 (in life date). Although the WIL study report presented some tabular data indicating ability to detect FOB changes, the study report does not meet present requirements for positive control data needed for a developmental neurotoxicity study. 1) There were no details on the animals used in the study. 2) There were no numerical data (individual animal and mean (SD) data) presented to support the conclusions of the investigator for Motor activity, FOB or Neuropathology. 3) There were no details on the statistical methods used.** This positive control study does not satisfy any guideline requirement.

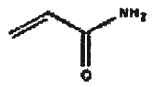
COMPLIANCE: No Quality Assurance or Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

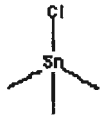
A. MATERIALS:

1. **Test material:** 1) Acrylamide; 2) Trimethyltin chloride

Description:	1); 2) Not described
Lot/batch #:	1); 2) Not described
Purity:	1) 99% ai; 2) not described
CAS # of TGAI:	1);79-06-1; 2) 1066-45-1
Structure:	1)



2)



2. **Vehicle:** 1) deionized water; 2) physiological saline

3. **Test animals:**

Species:	Rat	
Strain:	Not described	
Age/weight at dosing:	Not described	
Source:	Not described	
Housing:	Not described	
Diet:	Not described	
Water:	Not described	
Environmental conditions:	**Temperature:**	Not described
	Humidity:	Not described
	Air changes:	Not described
	Photoperiod:	Not described
Acclimation period:	Not reported	

B. STUDY DESIGN:

1. **In life dates:** Start: January 14, 1991; End: February 15, 1991

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1. The method of assignment was not specified.

TABLE 1. Study design					
Experimental parameter (test relative to dosing)	Acrylamide (mg/kg/day)[a]				Trimethyltin Cl (mg/kg)
	0	5	10	20	7.5
Number of Rats					
Total no. of animals assigned	12/sex	12/sex	12/sex	12/sex	5/sex
FOB (pre study; 1h,6h; Days 1, 7, 14, 21, 28)	12/sex	12/sex	12/sex	12/sex	--
Motor activity (pre; 28 days)	12/sex	12/sex	12/sex	12/sex	--
Neuropathology (at completion of testing)	12/sex	12/sex	12/sex	12/sex	5/sex

Data obtained from page 96, (MRID 45457501f)

[a] Doses given 5 days/wk/4 wks (route not specified)

3. **Dose selection rationale:** 1); 2) Doses of the reference material tested was selected based on values in the published literature. No details were provided.

4. **Dosage administration:** 1) Doses of **acrylamide** were given 5 days/wk for 4 weeks (route not specified) in deionized water (5 mL/kg) at 0, 5, 10 or 20 mg/kg/day.

   2) **Trimethyltin chloride** was administered intraperitoneally as a single dose in physiologic saline.

5. **Statistics:** Statistical analyses were performed but the methods were not described.

C. METHODS / OBSERVATIONS:

1. Neurobehavioral assessment – acrylamide only:

a. **Functional observational battery (FOB):** FOB tests were performed on the control and acrylamide-treated rats in a prestudy; then at 1 hr, 6 hr, and on Days 1, 7, 14, 21, and 28 after the first dose administration. The tests were performed using WIL Laboratory standard operating procedures (not provided in the study report). The observations conducted are given in Table 2.

TABLE 2. Functional observations					
X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture		Reactivity	X	Mobility
	Vocalization	X	Lacrimation*/chromodacryorrhea	X	Rearing
X	Convulsions	X	Salivation	X	Arousal/ gereral activity level
X	Tremors	X	Piloerection (open field)	X	Convulsions
X	Abnormal Movements	X	Fur appearance	X	Tremors
X	Palpebral closure	X	Palpebral closure (open field)	X	Abnormal movements
X	Feces consistency	X	Respiratory rate (open field)	X	Urination / defecation
X	Biting	X	Red/crusty deposits	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture
X	Approach response	X	Eye prominence (open field)	X	Gait score
X	Touch response	X	Muscle tone	X	Bizarre / stereotypic behaviour
X	Startle response	X	Ease of removal	X	Backing
X	Tail pinch response	X	Ease of handling	X	Time to first step
X	Pupil response				
X	Eyeblink response		PHYSIOLOGICAL OBSERVATIONS		NEUROMUSCULAR OBSERVATIONS
X	Forelimb extension	X	Body weight	X	Hindlimb extensor strength
X	Hindlimb extension	X	Body temperature	X	Forelimb grip strength
X	Air righting reflex	X	Catalepsy	X	Hindlimb grip strength
X	Olfactory orientation	X	Respiratory character	X	Hindlimb foot splay
	Auditory reaction		OTHER OBSERVATIONS	X	Rotarod performance
		X	Fur appearance		

b. **Motor activity:** Motor activity tests of control and acrylamide-treated rats (12/sex/group) were conducted in a prestudy and at 28 days after the first dose. No details of the testing were provided.

2. **Sacrifice and neuropathology – Acrylamide and trimethyltin chloride:** At the end of the study all animals were euthanized and subjected to perfusion fixation using 1.5% glutaraldehyde/4% formaldehyde solution. The following CHECKED (X) neurological tissues were collected and histological examination was conducted on twelve rats (6/sex/group) from the control and high dose groups.

X	BRAIN	X	OTHER
X	Forebrain	X	Trigeminal nerve/gasserian ganglion
X	Cerebrum, center	X	Peroneal nerve
X	Midbrain	X	Sciatic nerve (mid thigh)
X	Cerebellum and pons	X	Sciatic nerve (at sciatic notch)
X	Medulla oblongata	X	Sural nerve
	Spine	X	Tibial nerve
X	Spinal chord at swellings C_3-C_8 , and at lumbar swellings T_{13}-L_4	X	Optic nerve
X	Cervical dorsal root ganglion (C_3-C_8)	X	Eyes
X	Cervical ventral root fibers (C_3-C_8)		
X	Cervical dorsal root fibers (C_3-C_8)		
X	Lumbar dorsal root ganglion at T_{13}-L_4		
X	Lumbar dorsal root fibers at T_{13}-L_4		
X	Lumbar ventral root fibers at T_{13}-L_4		

II. RESULTS:

A. FUNCTIONAL OBSERVATIONAL BATTERY (FOB) - acrylamide:

Data were presented as dose group affected and no incidence rates were given. In the acrylamide-treated rats there were no remarkable differences relative to controls at the 1 hr, 6 hr, Day 1, or Day 7 testing intervals. Differences were observed in the 10 and 20 mg/kg/day treatment groups during the 14, 21, and 28 day evaluations, and became more apparent with each successive time point as evidence by the increased number of endpoints affected and the increased observation of the effect at both dose levels. Specific observations included alterations in muscle tone, startle response, tail pinch response, air righting reflex, reductions in hindlimb extensor strength, fore- and hindlimb grip strength, and rotarod performance; increased hind limb foot splay; and decreases in body temperature and body weight.

B. MOTOR ACTIVITY - acrylamide:

No data were presented in either tabular or graphical form. The study report states that there were no remarkable differences between the treated and control animals at the end of the 28-day treatment period. No explanation of this outcome was offered.

C. HISTOPATHOLOGY FINDINGS:

Acrylamide: No incidence data were presented. The study report states that biologically significant lesions indicative of neurotoxicity were observed in the acrylamide-treated rats (20 mg/kg/day) when compared to the control group. These included lesions of the trigeminal nerve (axonal degeneration, axonal digestion chambers, swollen axonal cylinders, and axonal demyelination), lumbar and dorsal root fibers, sural nerve, peronal nerve, lumbar root (females only), and cervical ventral root fibers (females only). Lesions noted in both males and females but statistically significant ($p<0.05$) in males only included lesions of the sciatic and tibial nerves.

Trimethyltin chloride: No incidence data were presented. The study report states that 2/5 (40%) male rats exhibited neuronal loss in the dentate gyrus. 1/5 (20%) male rats exhibited chromatolysis in the gasserian ganglion neurons.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The authors concluded that the procedures employed in the neurotoxicity screening battery at their laboratory are capable of detecting the primary neurotoxic effects produced by **acrylamide** and **trimethyltin chloride**.

B. REVIEWER COMMENTS:

No individual animal data were presented to evaluate, except a table on the groups affected (no incidence data) in the FOB. No individual or summary data were presented at all regarding the Motor activity and the neuropathology evaluations. The reviewer agrees with the investigators' conclusion relative to the FOB tests and the neuropathology evaluations. Insufficient methods and results were presented to evaluate the motor activity testing, and the authors offered no explanation for the apparent lack of detecting acrylamide-related effects in this testing.

WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting Motor Activity, FOB and neuropathology changes due to acrylamide treatment and neuropathology changes due to trimethyltin chloride (age of rats not specified). Although the WIL study report presented some tabular data indicating ability to detect FOB changes, the study report does not meet present requirements for positive control data needed for a developmental neurotoxicity study. The: 1) There were no details on the animals used in the study. 2) There were no numerical data (individual animal and mean (SD) data) presented to support the conclusions of the investigator for Motor activity, FOB or Neuropathology. 3) There were no details on the statistical methods used.

C. STUDY DEFICIENCIES:

The deficiencies were the absence of any details on the animals used in the study, the omission of motor activity and statistics methods, and the lack of any raw data and most of the summary data in the presentation of results.

DATA EVALUATION RECORD

STUDY TYPE: VALIDATION STUDY OF THE DIGISCAN MICRO ANIMAL MOTOR ACTIVITY SYSTEM (equipment no longer in use) by WIL RESEARCH LABS

MRID 45457501g

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
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Task Order No. 131-2006

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Quality Assurance:
Lee Ann Wilson, M.A.

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Date: MAY 11 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:** Marion Copley
Science Information Mgmt. Branch, HED (7509P) **Date:** 3/20/12
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature:**
Immediate Office, Health Effects Division (7509P) **Date:** 4/11/12
Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: Validation Study/Positive Control Data for Neurotoxicity Study (Motor Activity and Functional Observation Battery) - Rat

PC CODE: 1) 600091; 2) 600006 **DP BARCODE:** D302810

TEST MATERIAL (PURITY): 1) d-Amphetamine sulfate (99%); 2) Chlorpromazine hydrochloride (98%)

SYNONYMS: None

CITATION: Pitt, J. (2001). Positive and Historical Control Data for neurotoxicity Studies: WIL-99026: Validation Study of the Digiscan "Micro" Animal Motor Activity System (equipment no longer in use). Aventis Report No. WIL-2001-1; WIL Report No. WIL-99026. WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. June 26, 2001. MRID 45457501g (pp 1-5, 103-119). Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a motor activity positive control study (MRID 45457501g), four groups of 24 rats (12/sex/group) were administered single doses of 0, 0.5, 1.0 or 2.0 mg/kg d-amphetamine sulfate (ip injection) then tested 20 minutes and 24 hours post-treatment in a Digiscan Micro Animal Motor Activity System. Functional Observational Battery (FOB) testing was also performed 1, 6 and 24 hours after dosing. After a rest period of one day, the same rats were injected (ip) with single doses of 0, 2.5, 5.0, or 10 mg/kg chlorpromazine HCl and the affect on motor activity and FOB endpoints tested. Motor activity profiles for untreated male and female rats (16/sex) were measured during an entire 24 hr light-dark cycle.

d-Amphetamine sulfate-treated males and female rats demonstrated a dose-dependent increase in total and ambulatory motor activity counts as compared to controls at 20 minutes post-treatment, but not at 24 hrs. All increases were statistically significant for all three dose levels ($p<0.05$). d-Amphetamine sulfate treatment increased the number of rearing episodes in high dose males and altered posture in the mid-dose females during the 1-hour post-treatment evaluation.

Chlorpromazine HCl caused dose-dependent decreases in total and ambulatory activity counts in both males and females at 20 minutes post-treatment, but not at 24 hrs. Decreases were observed at all dose levels in male rats, but statistically significant ($p<0.05$) only at the mid-dose level. In females, the differences observed in total count were statistically significant for all three dose levels, but only at the mid- and high dose levels for the ambulatory counts. The FOB results were presented in tabular form as dose group affected, but without incidence data. Chlorpromazine HCl-treatment caused alterations in palpebral closure, respiratory rate, mobility, gait, gait score, and body temperature at 1 hr post-treatment.

Motor activity profiles The results were similar to those reported in the published literature. Reliability studies on the motor activity testing system were conducted on 48 rats/sex/group across four consecutive days using two separate devices. The devices appeared to give similar results as the standard deviations for each pairing overlapped substantially.

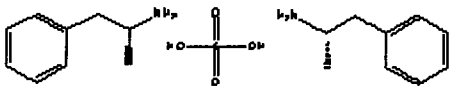
This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting changes in FOB and Motor Activity tests in rats (age, strain and source are unspecified) due to d-Amphetamine sulfate or Chlorpromazine HCl treatment for the time period around 1990 (in life period of study). Although the WIL study report presented some numerical and graphical data indicating ability to detect changes, the results could not be confirmed due to the limited data presented (see deficiency section for details).** This positive control study does not satisfy any guideline requirement.

COMPLIANCE: No Quality Assurance, GLP, or Data Confidentiality statements were provided.

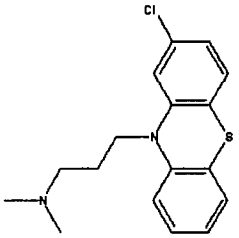
I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** d-Amphetamine sulfate

Description:	Not described
Lot/batch #:	Not described
Purity:	99%
CAS # of TGAI:	51-63-8
Structure:	

2. **Test material:** Chlorpromazine hydrochloride

Description:	Not described
Lot/batch #:	Not described
Purity:	98%
CAS # of TGAI:	69-09-0
Structure:	

3. **Vehicle:** Physiological saline was used as the vehicle.

4. **Test animals:**

Species:	Rat	
Strain:	Not described	
Age/weight at dosing:	Not described	
Source:	Not described	
Housing:	Not described	
Diet:	Not described	
Water:	Not described	
Environmental conditions:	**Temperature:**	72±4°F
	Humidity:	30-70%
	Air changes:	Not described
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	Not reported	

B. STUDY DESIGN:

1. **In life dates:** Study conducted between November 5, 1990 and November 30, 1990. Dates of dosing not provided.

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1. The method of assignment was not specified.

TABLE 1. Study design				
Experimental parameter (testing post dosing)	**D-amphetamine sulfate Dose (mg/kg)**			
	Control (0)	**0.5**	**1.0**	**2.0**
Total No. of animals assigned	24	24	24	24
Motor activity (pre, 20 min, 24 hrs) and FOB (pre, 1, 6, 24 hrs)	12/sex	12/sex	12/sex	12/sex
Experimental parameter	**Chlorpromazine HCl Dose (mg/kg)**			
	Control (0)	**2.5**	**5.0**	**10**
Total No. of animals assigned (same rats as for amphetamine)	24	24	24	24
Motor activity (20 min, 24 hrs) and FOB (1, 6, 24 hrs)	12/sex	12/sex	12/sex	12/sex
Experimental parameter	**Untreated**			
Total No. of animals assigned	128			
Rat strain profiling	16/sex			
Motor activity device reliability	48/sex			

Data obtained from pages 106-107 in the study report (MRID 45457501g)

3. **Dose selection rationale:** Doses of the reference materials tested were selected based on range finding studies (details not provided).

4. **Dosage administration:** Single doses of d-amphetamine sulfate were given to rats by intraperitoneal (ip) injection at 0.0 (1 mL/kg saline), 0.5, 1.0 or 2.0 mg/kg. Single doses of chlorpromazine HCl were given to rats by ip injection at 0.0 (1 mL/kg saline), 2.5, 5.0 or 10 mg/kg.

5. **Dosage preparation and analysis:** Solutions of the reference materials were prepared in saline such that each dose could be delivered in a volume of 1 mL/kg body weight. Analyses of the preparations for concentration, stability, and homogeneity were not addressed in the study report.

4. **Statistics:** Performed but the methods were not described.

C. METHODS / OBSERVATIONS:

1. **Neurobehavioral assessment**:

   a. **Motor activity: Pretreatment period:** All animals used in the study were tested for 61 minutes (the first minute of all sessions was not counted) to determine a habituation profile for untreated animals to the test chambers. This assured that asymptotic activity counts could be reached in controls for the last 20% of the test session. Based on this, a 41 minute period was determined to be optimum for testing following treatment.

   D-amphetamine sulfate treatment: Motor activity (ambulatory and stereotypic counts) of control and d-amphetamine sulfate-treated rats (12/sex/group) was conducted using a Digiscan Micro Animal Motor Activity System (Omnitech Electronics, Columbus, OH). Each unit was a clear acrylic chamber with 16 photobeams and two sensor panels. Activity was measured as interruptions of the photobeams, and the monitoring sequence was managed by software. Animals were tested either simultaneously (up to four) or

separately (stagger start). Data were collected and analyzed by a PC in 1-minute intervals and summed as 40-minute sessions and as four 10 minute subsessions. Ambulatory (gross movements) and stereotypic (fine motor movements) counts were accumulated separately with the combination of the two comprising total activity. Animals were allowed to acclimate for one minute before data acquisition commenced. Forty minute sessions were conducted at 20 minutes and 24 hrs post-treatment. Monitoring took place in a sound proof room equipped with a white-noise generator set to operate at approximately 70 db.

Chlorpromazine HCl treatment: Similarly, locomotor activity was measured on the same rats after a rest period of one day, except the animals were injected (ip) with 0, 2.5, 5.0, or 10 mg/kg chlorpromazine HCl. The d-amphetamine sulfate 24 hour test period was used instead of prestudy testing since the animals demonstrated normal behavior/activity.

Strain profiling: Male and female rats (16/sex) were monitored in the apparatus for 24 hrs on consecutive days (males on one day; females the other day) to obtain activity profiles on this strain of rat (not specified) and compared with profiles in published reports.

System reliability: The reliability of the testing system was evaluated by monitoring 48 rats/sex in three 41 minute sessions (16 rats/session) each day for 4 consecutive days. Animals were tested simultaneously and in the stagger start mode. The results were compared across days and two Digiscan Micro Animal Motor Activity System devices.

b. **Functional observational battery (FOB):** FOB tests were performed on the controls, d-amphetamine sulfate- and chlorpromazine HCl-treated rats (including controls) in a prestudy and at 1, 6, and 24 hours after administration of the reference compounds. The tests were performed using WIL Laboratory standard operating procedures (reported to be described in WIL-188003, study not available for review). The observations conducted are listed in Table 2.

	TABLE 2. FUNCTIONAL OBSERVATIONS
	Signs of autonomic function:
X	1) Lacrimation and salivation
X	2) Pupillary response to light
X	3) Palpebral closure
X	4) Defecation
X	5) Urination
X	6) Tremors
X	7) Biting
X	8) Feces consistency
X	9) Chromodacryorrhea
X	10) Piloerection
X	11) Respiratory rate
X	12) Respiratory character
X	13) Red deposits on eyes, nose, mouth
X	14) Crusty deposits on eyes, nose, mouth
X	15) Color of eyes, skin, mucous membranes
X	16) Eye prominence Muscle tone
X	17) Bizarre behavior
	Neuromuscular:
X	1) Time to first step
X	2) Gait score
X	3) Mobility score
X	4) Foot splay
X	5) Forelimb grip strength
X	6) Hindlimb grip strength
X	7) Air righting
X	8) Rotorod
X	9) Forelimb extension
X	10) Hindlimb extension
	Sensorimotor
X	1) Tail pinch
X	2) Startle response
X	3) Touch response
X	4) Approach response
X	5) Olfactory response
X	6) Eye blink response
	CNS excitability
X	1) Ease of removal
X	2) Handling reactivity
X	3) Clonic movements
X	4) Tonic movements
X	5) Arousal
	6) Vocalization
	CNS activity:
X	1) Posture
X	2) Catalepsy
X	3) Rearing
X	4) Grooming
X	5) Backing
	Physiological:
X	1) Body weight
X	2) Body temperature
X	3) Fur Appearance

2. **Positive controls**: This was a positive control study.

II. RESULTS –

A. NEUROBEHAVIORAL ASSESSMENT:

1. **Motor activity:** Data were presented graphically as mean ambulatory and total activity counts, separately for males and females, for each of the four dose levels for sessions conducted at 20 minutes and 24 hrs post-treatment. There were no individual animal data or means (SD) for the sessions or subsessions. There was little numerical information presented.

   D-amphetamine sulfate treatment: The graph shows dose-dependent increases in total and ambulatory activity counts compared to controls were observed in both sexes at 20 minutes post-treatment with d-amphetamine sulfate, but not at 24 hrs. The text states that at 20 minutes male values for ambulatory (total) counts were increased 165% (136%), 244% (211%), 273% (246%), low to high dose, respectively and females, 55% (53%), 84% (77%), 82% (84%), low to high dose, respectively. All increases were statistically significant in both males and females at all three dose levels ($p<0.05$).

   Chlorpromazine HCl treatment: Dose-dependent decreases in total and ambulatory activity counts compared to controls were observed in both males and females at 20 minutes post-treatment with chlorpromazine HCl, but not at 24 hrs. Decreases were observed at all dose levels in males as compared to controls, but statistically significant ($p<0.05$) only at the mid-dose level for ambulatory (total) activity counts as percent of controls as follows: 79% (75%), 42% (41%), 42% (46%), low to high dose, respectively. In females, the differences observed in total counts were statistically significant at all three dose levels, but only in the mid- and high dose levels for the ambulatory counts. Values for ambulatory (total) counts as percent of controls in the text of the report are: 67% (64%), 36% (39%), 17% (26%), low to high dose, respectively.

   Strain profiling: Data were only presented graphically as mean ambulatory and total activity counts, separately for males and females. The activity profiles for the male and female rats measured during the entire 24 hr light-dark cycle are similar to those reported in published literature1 (not provided in the study report).

   System reliability: The reliability data on the testing system evaluated across four consecutive days and two devices are shown in Table 3. The devices appear to give similar results as the standard deviations for each pairing overlap substantially, although no individual animal data or statistical analyses were provided.

1 Weir, P.J., Guerriero, F.J. and Walker, R.F. (1989) Implementation of a Primary Screen for Developmental Neurotoxicity. Fund. Appl. Toxicol. 13:118-136.

TABLE 3. Motor activity Reliability Study [activity counts ±S.D. (C.V.)]				
Test mode (Day)	Device 1 (total)	Device 2 (total)	Device 1 (ambulatory)	Device 2 (ambulatory)
Males				
Simultaneous (1)	835±413 (49.5%)	997±518 (51.9%)	NA	NA
Stagger (2)	1017±556 (54.6%)	1024±565 (55.1%)	NA	NA
Simultaneous (3)	NA	NA	537±282 (52.5%)	627±351 (55.9%)
Stagger (4)	NA	NA	641±380 (59.2%)	624±344 (55.1%)
Females				
Simultaneous (1)	1178±579 (49.1%)	1375±543 (39.4%)	NA	NA
Stagger (2)	1372±573 (41.7%)	1405±571 (40.6%)	NA	NA
Simultaneous (3)	NA	NA	717±357 (49.7%)	824±330 (40.0%)
Stagger (4)	NA	NA	859±377 (43.8%)	848±364 (42.9%)

Data were extracted from MRID 45457501g, page 119
Values represent mean ±s.d. (C.V.)
n= 16 rats/group

B. Functional observational battery (FOB):

Data were presented as dose group affected and no incidence rates or individual animal data were given. Therefore the results could not be confirmed.

In the **d-amphetamine sulfate**-treated rats there was an increase in the number of rearing episodes in high dose males and altered posture was noted in the mid-dose females when these groups were compared to controls during the 1-hour post-treatment evaluation.

In the **chlorpromazine HCl**-treated rats the differences were noted at the 1 hr post-treatment evaluation and to a lesser extent at the 6 hr evaluation. Both males and females in the mid- and high dose group showed alterations in palpebral closure, respiratory rate, mobility, gait, gait score, and body temperature. Many of these same changes were noted at the 6 hr post-treatment interval, but only at the high dose level. Other alterations (not specified in detail) generally seen only at the high dose level in both sexes 1 hr post-treatment were ease of handling, rearing episodes, arousal, rotarod performance, and catalepsy.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The authors concluded that the motor activity testing system employed at their laboratory is reliable and capable of detecting both increases and decreases in activity.

B. REVIEWER COMMENTS:

The reviewer agrees that the data presented appear to support the investigators' conclusions. However these data could not be confirmed (see deficiency section below). The studies on both the d-amphetamine sulfate- and chlorpromazine HCl-treated rats appear to demonstrate the ability to detect alterations in motor activity and the FOB. Other results appear show the reliability of the test system, and may confirm that the strain of rats used respond in a manner that is consistent with responses reported in published literature.

WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting changes in FOB and Motor Activity tests in rats (age, strain and source are unspecified) due to d-Amphetamine sulfate or Chlorpromazine HCl treatment for the time period around 1990 (in life period of study). Although the WIL study report presented some numerical and graphical data indicating ability to detect changes, the results could not be confirmed due to the limited data presented (see deficiency section for details). .

C. STUDY DEFICIENCIES: Due to the sketchy nature of this report, it is recommended that a complete report (including individual animal data) be submitted. This should include methods and statistical evaluation for all parameters evaluated.

Some examples of major deficiencies are:
1) the absence of any details on the animals used in the study (including strain, age, source)
2) the statistical methods were omitted.
3) the FOB methods are not present.
4) individual animal and summary data (including session and subsession data)

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:**
Science Information Mgmt. Branch, HED (7509P) **Date:** 3/20/12
EPA Secondary Reviewer: Jess Rowland **Signature:**
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 4/11/2

Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study (Positive Control) - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 600093 **DP BARCODE:** D302810

TEST MATERIAL (PURITY): Methimazole (% ai not provided)

SYNONYMS: 2-Mercapto-1-methylimidazole

CITATION: Schaefer, GJ (2006) Validation of Developmental Neurotoxicity Endpoints in Rats Administered Methimazole in Drinking Water. WIL Research Laboratories, Inc. WIL-99199, March 7, 2006. MRID 46779203. Unpublished.

SPONSOR: Nippon Soda Co., Ltd., 2-1, 2-Chome Ohtemachi, Chiyoda-ku, Tokyo 100-08165 Japan.

EXECUTIVE SUMMARY: In a positive control developmental neurotoxicity study (MRID 446779203) Methimazole (purity and lot # not provided) was administered to pregnant female Sprague-Dawley rats (#/dose not provided) in the drinking water at concentrations of 0, 10, 30, or 100 ppm from gestation day (GD) 6 through postnatal day (PND) 21. The report stated that body weights, food and water consumption, and a modified functional observational battery (mFOB) were evaluated in the dams. Brain weights, qualitative neuropathology and quantitative brain morphometry were evaluated in the pups at PND 11, 22 and 72. Pups were also evaluated for auditory startle, locomotor activity, learning and memory (Biel Maze) and mFOB several times. Details on these evaluation methods were not provided.

The investigators concluded that:

- Based on the endpoints affected (e.g., pup body weight, locomotor activity, auditory startle response, learning and memory, brain weight and length, qualitative and quantitative neuropathology), Methimazole altered multiple functional domains in the F1 generation demonstrating that this compound is acceptable for use as a positive control agent in DNT studies.

- Both immersion and perfusion fixation of the PND 11 pup brains provided adequate tissue samples for quantitative morphometrics and qualitative histopathology.

This study is classified **unacceptable/nonguideline. The reviewer can not determine whether WIL Research Laboratory can detect the effects discussed in the investigators' conclusions**

since there were no details regarding the methods, test animal, test material, individual animal data or summary data (other than several graphs). Based on the parameters in the graphs and those mentioned in the report's results section it appears that this study may be acceptable as positive control data if a complete report is submitted.

COMPLIANCE: The study was not subject to GLP. No Quality Assurance inspections were reported. A Data Confidentiality statement was provided.

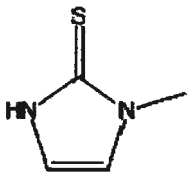
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Methimazole

Description:	Not provided
Lot #:	Not provided
Purity:	Not provided
Compound stability:	Not provided
CAS # of TGAI:	60-56-0

Structure:



2. Vehicle control:

3. Test animals (P):

Species:	Rat	
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR	
Age at study initiation:	Dam treated starting GD 6 (age not provided)	
Wt. at study initiation:	Not provided	
Source:	Charles River	
Housing:	Not provided	
Diet:	Not provided	
Water:	Not provided	
Environmental conditions:	Temperature:	Not provided
	Humidity:	Not provided
	Air changes:	Not provided
	Photoperiod:	Not provided
Acclimation period:	Not provided	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start and End: not provided (project completion date: March 7, 2006)

2. Study schedule: Methamizole substance was administered in the drinking water to the pregnant rats from gestation day 6 through postnatal day 21. Pups were evaluated using a modified functional observation battery (mFOB) (PND 4, 11, 21, 35, 45, and 60). They were evaluated for motor activity (on PND 13, 17, 21 and 61. Learning and memory (Biel water maze) was evaluated on PND 22 and 62. Neuropathology and morphometry were evaluated on PND 11, 22, 72.

3. Mating procedure: Information was not provided.

4. **Animal assignment:** There was no information provided about assignment of dams and pups to groups or how many animals were present in each group.

TABLE 1. Study design

Experimental parameter (PND)	Dose (ppm in drinking water)[a]			
	Control (0)	LDT (10)	MDT (30)	HDT (100)
Maternal animals				
No. of maternal animals assigned	NA	NA	NA	NA
Offspring				
mFOB (PND 4, 11, 21, 35, 45, 60)	NA	NA	NA	NA
Motor activity (PND 13, 17, 21, 61)	NA	NA	NA	NA
Learning and memory (PND 22, 62)	NA	NA	NA	NA
Brain neuropathology and morphometry (PND 11, 22, 72)	NA	NA	NA	NA

a from GD 6 through PND 21
NA – information is unavailable

5. **Dose selection rationale:** Not provided.

6. **Dosage administration:** Doses were administered maternal animals in the drinking water, on GD 6 through PND 10. No additional details were provided.

7. **Dosage preparation and analysis:** Not provided.

Results: Information regarding homogeneity, stability and concentration analyses, if obtained, were not provided.

C. **OBSERVATIONS:**

1. **In-life observations:**

a. **Maternal animals:** Not provided.

b. **Offspring:**

1.) **Litter observations:** Not provided.

2.) **Developmental landmarks:** Not provided.

3.) **Postweaning observations:** Not provided.

4.) **Neurobehavioral evaluations:** The test schedules are summarized as follows from the report. Detailed description of methods and endpoints was not provided.

i. **Functional observational battery (FOB):** On postnatal days PND 4, 11, 21, 35, 45, 60 pups were evaluated in an FOB assessment. No details were provided.

ii. **Motor activity testing:** Locomotor activity was evaluated on days 13, 17, 21, and

CH

61. No details were provided.

iii. **Auditory startle reflex habituation:** Auditory startle reflex testing was performed on PND 20 and 60. No details were provided.

iv. **Learning and memory testing:** Learning and memory testing was performed on PNDs 22 and 62 using a Biel water maze. No details were provided.

2. **Postmortem observations:**

a. **Maternal animals:** No details were provided.

b. **Offspring:** The report stated that: Qualitative neuropathology and quantitative morphometry evaluations were conducted at PND 11 (comparing immersion and perfusion fixation at PND 11), 22 and 72. For quantitative morphometry, 10 measurements were made on 3 levels. No additional details were provided.

D. DATA ANALYSIS:

1. **Statistical analyses:** No details were provided.

II. RESULTS:

Although the report listed results the parameters tested, these could not be verified since there was no presentation of detailed methods, individual animal data or summary data (other than several graphs).

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that: Based on the endpoints affected (e.g., pup body weight, locomotor activity, auditory startle response, learning and memory, brain weight and length, qualitative and quantitative neuropathology), Methimazole altered multiple functional domains in the F1 generation demonstrating that this compound is acceptable for use as a positive control agent in DNT studies.

Both immersion and perfusion fixation of the PND 11 pup brains provided adequate tissue samples for quantitative morphometrics and qualitative histopathology.

B. **REVIEWER COMMENTS: The reviewer can not determine whether WIL Research Laboratory can detect the effects discussed in the investigators' conclusions since there were no details regarding the methods, test animal, test material, individual animal data or summary data (other than several graphs). Based on the parameters in the graphs and those mentioned in the report's results section it appears that this study may be acceptable as positive control data if a complete report is submitted.**

C. STUDY DEFICIENCIES: See reviewer's comments.

DATA EVALUATION RECORD

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY (POSITIVE CONTROL)–RAT
DATA FROM WIL RESEARCH LABORATORIES, INC.
MRID 46779204 (formerly MRID 45457501h)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131-2006

Primary Reviewer: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	Sylvia S. Talmage APR 13 2006
Secondary Reviewers: Carol Wood, Ph.D., D.A.B.T.	Signature: Date:	Carol S. Wood APR 13 2006
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross APR 13 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L.A. Wilson APR 13 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature:**
Science Information Mgmt. Branch, HED (7509P) **Date:** 3/26/12
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature:**
Immediate Office, Health Effects Division (7509P) **Date:** 4/11/12

Template version 02/06

TXR#: 0054597

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study (Positive Control) - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 057810 **DP BARCODE:** D302810

TEST MATERIAL (PURITY): PTU (% ai not provided)

SYNONYMS: Propylthiouracil; 6-N-propyl-2-thiouracil

CITATION: Pitt, JA, MD Nemec, DG Stump et al. (2001) Positive and Historical Control Data for Neurotoxicity Studies: A validation study for developmental neurotoxicity endpoints at WIL Research Laboratories, Inc.: effect of propylthiouracil (PTU) on developmental neurotoxicity endpoints in Crl:CD®(SD)IGS BR rats (WIL-99126). WIL Research Laboratories, Inc., 1407 George Rd., Ashland, OH 44805. WIL-2001-1, June 26, 2001. MRID 46779204. Unpublished.

SPONSOR: Aventis CropScience, P.O. Box 12014, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709.

EXECUTIVE SUMMARY: In a positive control developmental neurotoxicity study (MRID 46779204) PTU (purity not provided, lot# 76H2500) was administered to 25 female Sprague-Dawley Crl:CD®(SD)IGS BR rats per dose by gavage at dose levels of 0, 3.8, 19, or 38 mg/kg bw/day from gestation day (GD) 6 through postnatal day (PND) 10. Other than body weight, observations on dams were not reported; dams were sacrificed on PND 21. On PND 4, litters were culled to eight pups, divided approximately equal between sexes. Developmental landmarks (balanopreputial separation in males and vaginal patency in females) were recorded. Neurobehavioral assessment including functional observational battery (FOB, tested on PNDs 20, 35, 45, and 60), auditory startle (tested on PND 22), locomotor activity (tested on PNDs 13, 17, 21, and 60), and learning and memory (tested in a Biel maze on PNDs 21 and 62) was performed on 10 offspring/sex/group. Thyroid function (T_3, T_4, and TSH levels) was evaluated on PNDs 4 and 28 (3-10 animals/sex/group). On PND 11, brain morphometry was performed on one pup/sex/litter from the control and 38 mg/kg/day group. Additional postmortem examinations were not performed. Details of most methods were not included.

Clinical data were not provided. Late in gestation, maternal body weight was significantly decreased in the 38 mg/kg/day group (data not reported).

Live litter size was significantly reduced in the 38 mg/kg/day group ($p<0.05$), and PND 1 body weight was significantly less in all treated groups ($p<0.05$ or $p<0.01$). Pup viability was significantly reduced from birth to PND 4 in the 19 and 38 mg/kg/day groups (both $p<0.01$). Body weight of all treated groups remained less than controls through the study (data presented graphically). Sexual maturation was delayed in mid- and high-dose males by 3-4 days and in all treated females by 6-8 days.

FOB data were presented as dose group affected, and incidence rates were not given. Deficits were found in autonomic, neuromuscular, sensorimotor, CNS activity, and physiological measurements on PND 20 in males and females of all dose groups (no statistical analysis). All findings were generally resolved by PND 35 with the exception of decreases in forelimb and hindlimb grip strength and body weight measurements which persisted through PND 60.

Data for locomotor activity were presented graphically, as percentage of control value; interval data were not provided. Activity was increased for all treated males on PND 21 and in females on PNDs 17 and 21. However, no trends in dose or time were apparent for either sex. Data were insufficient to observe habituation.

Mean values for auditory startle response were presented graphically; interval data were not provided. On PND 22, the peak auditory startle response was significantly reduced and the latency to peak response was significantly increased for both sexes at all dosage levels (all $p<0.01$). A dose-response was apparent for peak response, but not for latency to peak response. Habituation could not be determined from the data.

Swimming ability in the Biel maze was greatly reduced in a dose-dependent manner in all treated males and females on PND 21 and corresponded with an increase in latency to escape ($p<0.05$ or $p<0.01$). No differences in swimming ability were evident on PND 62, but latency to escape was increased equally in treated groups of each sex (all $p<0.01$).

For thyroid function, the report stated that "serum T4 levels were reduced for each dose group on PND 4 for both sexes combined and on PND 28 for both sexes separately. Serum TSH levels were reduced at all dosage levels on PND 4 and serum T3 levels were reduced for each male dose group on PND 28, but not for females (data not shown)."

Mean values were presented for 35 unidentified brain measurements. Significant differences between the control and 38 mg/kg/day groups were listed separately (e.g., the vertical thickness of the frontoparietal cortex on coronal Level I in males and females), but the list was not matched with the morphometry data to indicate whether the changes were increases or decreases.

This study is classified **Unacceptable/Non-guideline. WIL Research Laboratory has not demonstrated (due to reporting deficiencies) proficiency in this study for detecting neurotoxicity changes due to PTU treatment in pre- and postweaning Crl:CD® (SD)IGS BR rat pups (time period not provided). Although WIL Research Laboratory staff did identify some developmental and behavioral deficits (FOB, motor activity, auditory startle, and learning and memory tests) the study report does not meet present OPPTS 870.6300 guidelines. The: 1) in life dates of the study were absent, 2) protocol details very limited, and 3) raw data were limited (sometimes only presented graphically). These reporting**

deficiencies limit the usefulness of the study to validate DNT studies. Presentation of the study dates, additional protocol detail (for example, details of the methods and scoring criteria for the FOB) and raw data for this study are necessary in order to confirm that the laboratory has proficiency for detecting the changes. This positive control study does not satisfy any guideline requirement.

COMPLIANCE: The study was not subject to GLP, but was conducted in general accordance with GLP standards. No Quality Assurance inspections were performed. A Data Confidentiality statement was provided.

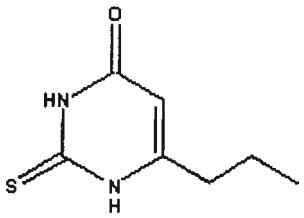
I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Propylthiouracil (PTU)

Description:	Not provided
Lot #:	76H2500, Sigma Aldrich, St. Louis, MO
Purity:	Not provided
Compound stability:	Not provided
CAS # of TGAI:	51-52-5

Structure:



2. **Vehicle control:** 0.5% Methylcellulose (lot# 97H0980; Sigma Chemical Co.)

3. **Test animals (P):**

Species:	Rat
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR
Age at study initiation:	Not provided
Wt. at study initiation:	Not provided
Source:	Charles River Breeding Laboratories, Raleigh, NC
Housing:	Individually with litters in plastic maternity cages with Bed-O-Cobs® nesting material to PND 28; then pups 2-3/dose/sex; 1 pup/cage after PND 35
Diet:	PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002, *ad libitum*
Water:	Reverse osmosis-purified water, *ad libitum*
Environmental conditions:	**Temperature:** 72±4°F **Humidity:** 30-70% **Air changes:** 10/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	Not provided

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: not provided; End: not provided (project completion date: June 26, 2001)

2. **Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals by oral gavage from gestation day 6 through postnatal day 10. Pups were weaned on postnatal day 21, at which time maternal animals were killed. F_1 pups remained on study until at least postnatal day 77 (study termination).

3. **Mating procedure:** Females were paired 1:1 with resident males (strain and source not provided). Each female was examined daily during the mating period to identify sperm cells

in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0. After successful mating, each pregnant female was placed into an individual plastic cage with a solid bottom and bedding, where it was maintained through gestation and lactation.

4. **Animal assignment:** Mated females were assigned to dose groups as indicated in Table 1. The basis for assignment was not provided. Dams were not tested for neurotoxicity.

   Offspring were assigned to testing subgroups at the time of litter standardization on postnatal day 4 (Table 1). Pups were randomized into treatment groups and then assigned to testing groups within each treatment group. Separate, dedicated groups of 10 offspring/sex/group were assigned to each of the test groups in Table 1 and were evaluated at each time point. Separate groups of animals were used for learning and memory tests on PNDs 21 and 62. The method of randomization was not provided. Specific details on randomization among litters was stated only for brain morphometry, i.e., one pup/sex/litter.

TABLE 1. Study design

Experimental parameter (PND)	Dose (mg/kg/day)			
	Control (0)	LDT (3.8)	MDT (19)	HDT (38)
Maternal animals				
No. of maternal animals assigned	25	25	25	25
Offspring				
Detailed clinical/FOB (PND 20, 35, 45, 60)	10/sex	10/sex	10/sex	10/sex
Motor activity (PND 13, 17, 21, 60)	10/sex	10/sex	10/sex	10/sex
Auditory startle habituation (PND 22)	10/sex	10/sex	10/sex	10/sex
Learning and memory (PND 21, 62)	10/sex	10/sex	10/sex	10/sex
Brain morphometry (PND 11)	25/sex	—	—	25/sex
Serum T_3, T_4, and TSH levels (PND 4, 28)	3-10/sex	3-10/sex	3-10/sex	3-10/sex

5. **Dose selection rationale:** The basis for selection of dose levels was not provided. PTU is known to produce hypothyroidism which, in turn, produces behavioral deficits.

6. **Dosage administration:** All doses were administered once daily to maternal animals by gavage, on GD 6 through PND 10, in a volume of 12.5 mL/kg of body weight/day. Dosing was based on the most recent body weight determination (not provided).

7. **Dosage preparation and analysis:** Formulations were prepared weekly by mixing appropriate amounts of test substance with 0.5% methylcellulose. Storage conditions were not described. Analyses for stability, homogeneity, and concentration of the test substance in methylcellulose were not provided.

Results:

Homogeneity analysis: Data were not provided

Stability analysis: Data were not provided

Concentration analysis: Data were not provided

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Daily checks for mortality, moribundity, and clinical signs of toxicity were conducted on maternal animals. Dams were not tested for neurotoxicity.

Individual maternal body weight and food consumption data were recorded twice weekly from GD 0 through PND 21.

b. **Offspring:**

1.) **Litter observations:** Live pups were counted, sexed and weighed individually for each litter on postnatal days 1, 4, 7, 11, 17, and 21. No additional examinations were described.

On day 4 postpartum, litters were standardized (procedure not described) to a maximum of 8 pups/litter (3-5/sex/litter, as nearly as possible); excess pups were killed and discarded. There was no mentioned of discarded litters due to small numbers of pups.

2.) **Developmental landmarks:** Anogenital distance was measured for all offspring on PND 1. Male offspring were examined daily for balanopreputial separation, and female offspring were examined daily for vaginal patency. The age of onset was recorded. Pups were weighed at this time.

3.) **Postweaning observations:** Other than the FOB, postweaning observations were not described. Individual offspring body weight data were recorded weekly (PND 28, 35, 42, 49, 56, 63, 70, and 77).

4.) **Neurobehavioral evaluations:** Testing was performed without knowledge of the animal group. Observations and the schedule for those observations are summarized as follows from the report. Detailed description of endpoints was not provided.

i. **Functional observational battery (FOB):** On postnatal days 20, 35, 45, and 60 (±2), a total of 10 offspring/sex/group (randomization from litters was not described) was examined outside the home cage in an FOB assessment. The same group of animals was utilized at all test intervals. The study authors stated that, whenever possible, testing was performed by the same trained technicians who were blind to the animal's group assignment. The study was performed before the development of U.S. EPA's standard FOB, but utilized previously developed protocols (see Table below; references were cited).

	FUNCTIONAL OBSERVATIONS
	Signs of autonomic function, including:
X	1) Lacrimation and salivation
X	2) Pupillary response to light
X	3) Palpebral closure
X	4) Defecation
X	5) Urination
	Neuromuscular:
X	1) Time to first step
X	2) Gait score
X	3) Mobility score
X	4) Foot splay
X	5) Forelimb grip strength
X	6) Hindlimb grip strength
X	7) Air righting
X	8) Rotorod
	Sensorimotor
X	1) Tail pinch
X	2) Startle response
X	3) touch response
X	4) Approach response
X	5) Olfactory response
	CNS excitability
X	1) Ease of removal
X	2) Handling reactivity
X	3) Clonic movements
X	4) Tonic movements
X	5) Arousal
X	6) Vocalization
	CNS activity:
X	1) Posture
X	2) Catalepsy
X	3) Rearing
X	4) Grooming
X	5) Backing
	Physiological:
X	1) Body weight
X	2) Body temperature
X	3) Appearance

ii. **Motor activity testing:** Locomotor activity was evaluated in a dedicated group of 10 rats/sex/dose on days 13, 17, 21, and 60±2. Testing of treatment groups was balanced across test times. Locomotor activity was measured using a Digiscan Micro Animal Activity System (Omnitech Electronics Inc., Columbus, OH). The session was 41 minutes, with intervals of 1 minute.

iii. **Auditory startle reflex habituation:** Auditory startle reflex testing was performed on a dedicated group of 10 offspring/sex/dose on postnatal day 22. Instrumentation consisted of an Auditory Startle Response System (Coulbourn Instruments, Inc., Lehigh Valley, PA). The test session consisted of a 5-minute acclimation period with a background noise level of 70 db, followed by 50 presentations of a 50-ms 120 db noise burst given at 8-second intervals. Mean amplitude on each block (1-5) of 10 trials was recorded for each animal. Testing of latency and observation of habituation were not described.

iv. **Learning and memory testing:** Learning and memory testing was performed in dedicated groups of 10 offspring/sex/dose on PNDs 21 (±4) and 62 (±4). Different animals were tested at each interval. Using a water-filled multiple-T Biel maze with an escape ladder, animals were tested for time to escape and number of errors (deviates from the correct channel with all four feet). Animals were allowed three minutes to escape from the maze before removal. The minimum intertrial interval was one hour. No further information on environmental conditions was provided. The 7-day test consisted of the following:

Day 1:	each animal is given 4 trials to escape from a straight channel.
Day 2:	each animal is given 2 trials per day in Path A.
Days 4, 5, 6:	each animal is given 2 trials per day in Path B (reverse of Path A).
Day 7:	each animal is given 2 trials in Path A.

5.) **Thyroid function:** Serum T_3, T_4, and TSH levels were measured on 3-10 animals/sex/group on PNDs 4 and 28. No information was provided on blood collection or analyses methods.

6.) **Pharmacokinetic data:** Pharmacokinetic data were not collected.

2. **Postmortem observations:**

a. **Maternal animals:** Maternal animals were euthanized (method not provided) on PND 21 and discarded without postmortem examination.

b. **Offspring:** The offspring selected for brain morphometry (one pup/sex/litter of the control and 38 mg/kg/day dose groups) were sacrificed on postnatal day 11. Thirty-five linear measurements were made on four coronal sections of the cerebellum and midbrain and one sagittal section of the cerebellum and brainstem. Brain sections and morphometry measurements were not further described. Measurements were compiled from images scanned by computer. Measurement means were compared using an unpaired t-test. Brains were not weighed, and brain tissues were not examined for pathological lesions.

D. DATA ANALYSIS:

1. **Statistical analyses:** All analyses (body weight, hormone concentrations, survival, developmental landmarks, litter size, locomotor activity, startle response, and learning and memory) were two-tailed for a significance level of 1 and 5% by a one-way analysis of variance (ANOVA). If significant treatment effects were observed at a given time-point, then Dunnett's test was conducted to determine significant treatment differences from the control group. Descriptive FOB parameters were analyzed by Fisher's Exact Test. The text states that pups were randomized into treatment groups, but only the brain morphometry part of the study mentions animal assignment related to litter. These methods of analysis appear adequate.

2. **Indices:**

   a. **Reproductive indices:** Mean values were presented on number born and live litter size.

   b. **Offspring viability indices:** Mean values were presented on percent males born and percent survival per litter (birth to PND 4 and PND 4 to weaning).

3. **Positive and historical control data:** This was a positive control study included in a packet of positive control studies performed by WIL Research Laboratories.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** Clinical signs and mortality data were not presented for maternal animals.

2. **Body weight and food consumption:** The study authors stated that maternal body weight was significantly decreased in the 38 mg/kg/day group late in gestation, but the data were not shown. Food consumption values for pregnant or nursing dams were not presented.

3. **Test substance intake:** Administration was via gavage.

4. **Reproductive performance:** Twenty-five females/dose group were mated. The number of litters, intercurrent deaths, mean gestation duration, and incidence of dystocia were not reported.

5. **Maternal postmortem results:** Postmortem examinations were not performed.

B. OFFSPRING:

1. **Viability and clinical signs:** Litter size and viability (survival) results of pups during lactation are summarized from the report in Table 2. Clinical observations on offspring were not reported.

TABLE 2. Litter size and viability (±SD) [a]				
Observation	Dose (mg/kg/day)			
	Control (0)	LDT (3.8)	MDT (19)	HDT (38)
Total number born	15.1±1.5	15.0±1.4	14.1±3.3	13.5±2.3
Number born live	14.2±3.4	14.6±2.3	12.9±4.4	11.4±5.2*
Number born dead	NR	NR	NR	NR
Sex Ratio Day 0 (% %)	52.2±12.6	53.0±11.5	50.1±16.7	44.3±15.1
Viability index (%)	88.1±24.8	91.8±20.2	77.4±28.8**	69.4±35.9**
Lactation index (%)	98.2±4.8	96.3±9.8	95.4±12.6	89.9±24.6
Mean pup weight, PND 1				
Males	6.5±0.7	5.8±0.6**	5.8±0.5**	5.7±0.6**
Females	6.1±0.7	5.7±0.5*	5.5±0.4**	5.6±0.5*

[a] Data obtained from page 11, MRID 46779204.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.
NR = not reported

2. **Body weight:** Except for PND 1, offspring body weight during lactation was presented graphically. Male and female offspring body weights on PND 1 are listed in Table 2. Mean weight for males was statistically significantly lower than the control by 11-12% in all treatment groups (all $p<0.01$), but did not show a dose-response relationship. Male pup weight remained reduced throughout the lactation period. On PND 21, all treated male groups weighed ≥10 g less than the control group (all $p<0.01$).

   On PND 1, treated females weighed significantly less than the control group by 7-10% ($p<0.05$ or $p<0.01$), but there was no evidence of a dose-response among treatment groups. Female pup weight remained reduced throughout the lactation period. On PND 21, all treated female groups weighed ≥10 g less than the control group (all $p<0.01$).

   The graphical data indicate that for each group, body weight before and after culling on PND 4 were approximately the same.

   Offspring postweaning body weight of treated groups remained reduced for the duration of the study (data presented graphically). At study termination on PND 77, differences between the male control and treated groups were statistically significant (all $p<0.01$); statistical significance for females was reported only in the 19 mg/kg/day dose group ($p<0.01$). From PND 28 to PND 77, the weight difference between the male control and treated groups widened, whereas that of females did not.

3. **Developmental landmarks:**

   a. **Sexual maturation:** In males, the appearance of **preputial separation** was significantly delayed by 3-4 days in the 19 ($p<0.01$) and 38 mg/kg/day dose groups ($p<0.05$). **Vaginal patency** in females was significantly delayed by 6.5-8 days in all treated groups (all $p<0.01$). The mean body weight at which these developmental landmarks appeared was

provided. The increased weight in the females indicates that the delay in vaginal opening was not due to a delay in weight gain. The data are presented in Table 3.

TABLE 3. Mean (±SD) age of sexual maturation (PND) [a]

Parameter	Dose (mg/kg/day)			
	Control (0)	LDT (3.8)	MDT (19)	HDT (38)
N (M/F)[b]				
Preputial separation (males)	45.1±3.0	46.6±2.8	49.5±4.0**	48.1±2.4*
(body weight)	(212±16.2)	(188±21.9)**	(194±14.3**)	(190±14.7**)
Vaginal opening (females)	33.4±2.8	39.8±2.5**	41.5±3.0**	41.4±2.4**
(body weight)	(101±9.0)	(113±15.4**)	(111±11.6*)	(110±15.6)

[a] Data obtained from page 11, MRID 46779204

[b] Measured for all live pups.

* Statistically different from control, $p<0.05$.

** Statistically different from control, $p<0.01$.

b. **Physical landmarks:** Additional physical landmarks were not measured.

4. **Behavioral assessments:**

a. **Functional observational battery:** Data were presented as dose group affected; incidence rates were not given. Deficits were found on PND 20 in males and females of all dose groups in **autonomic** (pupil response, palpebral closure), **neuromuscular** (gait score [females], mobility score [females], foot splay, forelimb and hindlimb grip strength, air righting, and rotorod), **sensorimotor** (startle response), **CNS activity** (catalepsy [high dose], rearing [females] and grooming [females]), and **physiological measurements** (body weight and body temperature). All findings were generally resolved by PND 35 with the exception of decreases in forelimb and hindlimb grip strength and body weight which persisted through PND 60. Males had a deficit in rearing only on PNDs 45 and 60.

b. **Locomotor activity:** Data for locomotor (overall) activity were presented graphically, as percentage of control value; interval data were not provided. Activity was increased in all treated males on PND 21 and in females on PNDs 17 and 21. Mid-dose groups appeared to have the greatest increase in activity compared with the controls, e.g., statistical significance ($p<0.05$) was attained for males only in the 19 mg/kg/day dose group on PND 21. No trends with dose or time were apparent for either sex. Data were insufficient to observe habituation.

c. **Auditory startle reflex habituation:** Mean values were presented graphically; interval data were not provided. On PND 22, the peak auditory startle response was significantly reduced and the latency to peak response was significantly increased for both sexes at all dosage levels (all $p<0.01$). A dose-response was apparent for peak response, but not for latency to peak response. Data were insufficient to observe habituation.

d. **Learning and memory testing:** On PND 21, swimming ability was greatly reduced in all treated male and female groups (Table 4) which corresponded with increased latency

to escape on PND 22. Data on latency to escape and number of errors were presented graphically for PNDs 22 and 62. On PND 22, latency to escape the Biel maze was significantly increased for both sexes in all dosage groups, generally in a dose-response manner ($p<0.05$ for males in the 3.8 mg/kg/day dose group; $p<0.01$ for both sexes in all other dose groups). No differences in swimming ability were evident in either sex on PND 61. Although swimming ability was not affected on PND 61, latency to escape was increased in both sexes in all dosage groups. The mean number of errors was not affected on PND 22, but was increased in all dosage groups on PND 66. No statistical analysis was performed on the mean number of errors.

TABLE 4. Biel swimming trials, swimming ability, test day 1 (mean seconds ± S.D.)[a,b]

Sex	Dose (mg/kg/day)			
	Control (0)	LDT (3.8)	MDT (19)	HDT (38)
PND 21				
Males	15.8±10.7	52.6±55.5	66.3±65.6	56.1±68.9
Females	14.3±9.2	46.3±46.8	86.0±72.3*	60.2±71.5
PND 61				
Males	7.0±2.3	8.9±2.7	8.6±3.9	9.6±5.1
Females	8.3±3.4	7.8±1.5	7.5±1.9	8.4±2.4

[a] Data obtained from page 11, MRID 46779204

[b] Mean of four trials to escape from a straight channel.

N = 10/sex/group.

* Statistically different from control, $p<0.05$.

5. **Thyroid function:** Data were not provided. Textual description follows. "Serum T_4 levels were reduced for each dose group on PND 4 for both sexes combined and on PND 28 for both sexes separately. Serum TSH levels were reduced at all dosage levels on PND 4 and serum T_3 levels were reduced for each male dose group on PND 28, but not for females (data not shown)."

6. **Postmortem results:**

   a. **Brain weights:** Brains were not weighed.

   b. **Neuropathology:** Macroscopic and microscopic examinations were not performed. Morphometric evaluations were presented as mean values for each sex for 35 different measurements (locations unspecified). The only information provided was a list of brain areas which differed morphometrically in treated and control animals: "These included the height of the hemisphere (females) and the vertical thickness of the frontoparietal cortex (females and males) on coronal Level I; the vertical distances between the layers of pyramidal neurons (males) and between the limbs of the dentate gyrus of the hippocampus (females and males), and the vertical and radial thicknesses of the frontoparietal cortex in females on coronal Level III; and the thickness of the base of cerebellar lobule 9 (females) and the thickness of the pons (females) on mid-sagittal Level V."

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that WIL Research laboratories can detect effects of PTU, a known developmental neurotoxicant, according to OPPTS Guideline 870.6300, developmental neurotoxicity studies. They demonstrated proficiency in identifying developmental endpoints such as reduced live litter size, low pup weight, and increased time to attain developmental landmarks. They demonstrated proficiency in performing behavioral assessments including decreases in grip strength, reduced acoustic startle response, reduced swimming ability, and increased time to escape the Biel maze.

B. **REVIEWER COMMENTS:** The reviewer agrees that WIL Research Laboratory staff did identify some developmental and behavioral deficits. In offspring, decreases in body weight, delayed developmental landmarks, reduced thyroid function, and deficits in FOB, motor activity, startle response, and learning and memory were clearly treatment related. However, the: 1) in life dates of the study were absent, 2) protocol details very limited, and 3) raw data were limited (sometimes only presented graphically). Therefore, the study does not meet present OPPTS 870.6300 guidelines. These deficiencies limit the usefulness of the study to validate DNT studies. Presentation of the study dates, additional protocol detail and raw data for this study are necessary in order to confirm that the laboratory has proficiency for detecting the changes.

   The pronounced effect on body weight is clearly a result of altered thyroid function. Because reduced growth could cause the resulting functional deficits, e.g., decreased swimming ability leading to increased latency to escape, the results may not represent actual developmental neurotoxicity in preweaning or young adult rats. This, however, does not eliminate the use of PTU as a positive control for demonstrating proficiency in conducting neurobehavioral tests.

C. **STUDY DEFICIENCIES:** The major reporting deficiencies were the absence of details in the methodology and the absence of raw data in the results section.